STUDIES IN THE AUSTRALIAN ACACIAS. IV.

THE LIFE HISTORY OF ACACIA BAILEYANA F.V.M.

Part 2. Gametophytes, Fertilization, Seed Production and Germination, and General Conclusion.

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(Plate viii; and sixty-six Text-figures.)

[Read 26th September, 1934.]

Introduction.

The first two papers of this enquiry (Newman, 1933*b* and 1934) described the ecology, habit, floral structures, reproductive processes as far as the production of the spores, and the chromosome numbers. The present paper completes the description of the reproductive processes.

The statement and correlation of times for the various stages of development will be reserved generally for a special section at the end of the paper. Annotations for the figures have been standardized and will be found, except for certain special cases, in the section "Notes on the Illustrations" (p. 312).

The material on which the study of the gametophytes is based was collected from trees in cultivation at Strathfield, Sydney; that for the fertilization and post-fertilization stages was collected from trees in cultivation at Hill Top,* except that the stages about the formation of cotyledons were in material from Pennant Hills, Sydney, and full-sized seeds were from Canberra, F.C.T. The illustrations of the germination of the microspore and three of those of the germination of the megaspore were made from slides that had been prepared when the writer was a post-graduate student at King's College, London, under Professor R. R. Gates, F.R.S.

The material collected at Hill Top and Pennant Hills was subjected to a certain degree of mutilation to facilitate the penetration of the fixing fluids. It was found that alcoholic fluids were more satisfactory from the point of view of sectioning. Material for non-alcoholic fluids was first rinsed in 90% alcohol and then water.

The following fixatives were used:

A. 1–2% Chromo-Acetic acid with 2 c.c. of 1% Osmic acid.
B. 4–5–5–50% Mercuric chloride–Acetic acid–Formalin–Alcohol at 38°C.
D. 6–50% Formalin–Alcohol.
E. 6% Formalin in Absolute Alcohol.

* I would express my thanks to Mr. E. Cheel, Government Botanist, for permission to collect this material on his property.

Of these fluids A and D were used the most, D being especially serviceable.

The stains used were Haidenhain's Iron Alum Haematoxylin differentiated with Acid Alcohol, and Safranin combined variously with two of Orange G, Light Green, and Gentian Violet in water or Clove Oil solution. Good results were obtained by differentiating Safranin with 0·02% Light Green in 95% Alcohol.

The Gametophytes.

male.

Germination of the Spore.

In examining the illustrations of microspores it must be remembered that their shape is determined by their position in the pollinium, and that the thickened extine is deposited on only one face of the spore—that towards the outside of the pollinium. In the fully formed microspore the cytoplasm is evenly and loosely vacuolate, and the chromatin of the nucleus is in a loose reticulum (Text-fig. 1). The thickened extine is sculptured by a rectangular groove (Text-figs. 2, 3; Plate viii, fig. 3), which has no connection with the emergence of the pollen tube. There is a thickening of the intine at the corners of the spore (Text-figs. 1–4), which has no apparent connection with germination.

The microspore germinates in the anther. The first sign is the appearance of large vacuoles against the walls of the spore except the outer wall (Text-fig. 2A), causing the spindle to be perpendicular and nearer to that wall. The division of the nucleus, thus orientated, takes place quite normally (Text-fig. 2, from A to F) ending with the formation of the small generative cell against the outer wall of the spore. The cytoplasm becomes uniformly and finely vacuolate and the generative cell is freed from the wall. The spore can now be regarded as the pollen grain (Text-fig. 3). The tube nucleus has a coarse reticulate chromatin, and the generative nucleus has the chromosomes organized and showing the split for the next division—probably the telophase condition.

The ripe pollen contains the spindle-shaped generative cell, whose nucleus shows chromosomes, and the tube nucleus which is usually partly disorganized and sometimes unidentifiable (Text-figs. 4–7, 5–9; Plate viii, fig. 2). The stains are taken so deeply by the pollen grains that it is very difficult to examine the generative cell for the presence of a definite membrane. The nucleus is certainly surrounded by an area of lighter cytoplasm, but it has not always been possible to demonstrate a membrane satisfactorily (cf. Text-fig. 4). The generative nucleus is undivided at the time of dehiscence of the anther.

Attention has already been called to the initiation of germination of the microspore by the formation of large vacuoles which orientate the spindle, and to the somewhat similar criterion of germination of the megaspore established by Rutgers in 1923 (Rutgers, 1923, p. 21, and Newman, 1929, p. 417). Sax and Edmonds (1933, p. 158) describe this phenomenon in detail in *Tradescantia*. Where adequate figures are shown in the literature, this vacuolation can usually be observed, even if it is not referred to. Among cases where it is to be observed may be mentioned, *Albizzia lebbek* (Maheshwari, 1931, Figs. 7–8), *Grevillea robusta* (Brough, 1933, Text-fig. 54), *Myricaria germanica* (Frisendahl, 1912, Figs. 38–44). After recording different phases of spindle formation in the division of the microspore of *Myricaria germanica*, Frisendahl (loc. cit., p. 27) suggests the evolution of the broad (non-converging) from the pointed spindle. In this direction then, *A. Baileyana* is constant with broad-ended spindles and is not primitive. In another pollinium-forming plant, *Asclepias cornuti*, Finn

(1925, Pl. 1, fig. 6) shows the anaphase split of the chromosomes in the division of the generative cell (so clearly shown in *Acacia Baileyana* in the division of the microspore nucleus), but interprets it as a peculiar form of the chromosome (loc. cit., p. 9).

There is considerable difference of opinion on the question of the presence or absence of a membrane enclosing a generative *cell.* A definite cell is recorded by Welsford (1914, p. 266) in *Lilium auratum,* and by Frisendahl (1912, p. 29) in *Myricaria germanica.* In *Asclepias cornuti,* Gager (1902, p. 137) describes a generative cell, and Finn (1925, p. 8) describes only a variably staining layer of pollen grain cytoplasm round the generative nucleus. In *Albizzia lebbek* (Maheshwari, 1931, p. 246) and *Cypripedium* (Pace, 1907, p. 358) a wall is formed but later disappears, the nuclei lying free in the pollen grain. Reeves (1930*a*, p. 36) describes a differentiated layer of cytoplasm round the generative nucleus in *Medicago sativa. Acacia Baileyana* agrees with the majority of plants described, in the spindle shape of the generative nucleus and associated cytoplasm. But it differs from many, such as *Lilium auratum* (Welsford, 1914, p. 265), in that the cytoplasm around the generative nucleus is less dense than that of the pollen grain.

Pollination.

Functional proterogyny has not been demonstrated, though the manner of anthesis suggests it (Newman, 1934, Plate vii, fig. 1). The opinion was formed after culture experiments that the pollen is at its best for germination about three or four days after anthesis. This might make proterogyny effective for any one flower-head, but would not serve in respect of any one tree in view of the great mass of flowers and the length of time during which anthesis is occurring on the tree. And, moreover, self pollination has been successfully carried out to produce a normal degree of pod formation, with the raising of normal seedlings. The mode of pollination is probably not specialized. In the nearly related *Albizzia lebbek,* Maheshwari (1931, p. 249) found no precise mechanism for pollination.

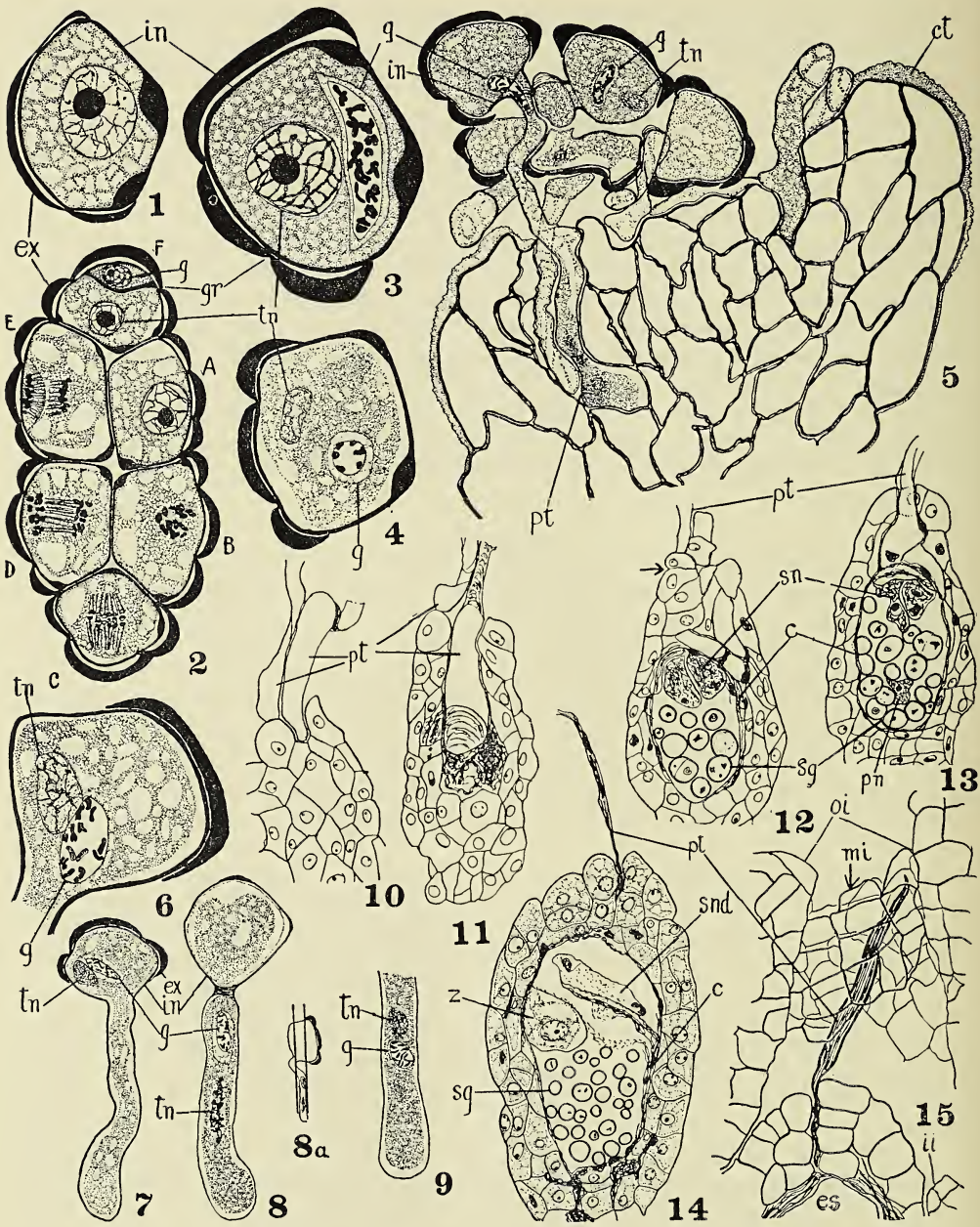
The pollinium lodges in the cup of the stigma. It must be held there by some adhesive substance; for there is no friction against hairs to overcome the reaction to the force required for the penetrating of the stigma by the many pollen tubes. In several instances two pollinia were seen on one stigma (Text-fig. 5 and Plate viii, fig. 3), and in one instance there were three.

The period of pollination and fertilization is marked by heavy frosts and cold winds, especially at Hill Top and Canberra. On many days, however, there would be bright sunshine. Seasonally this period is the depth of winter! And the writer has seen trees of another species of *Acacia* in full flower covered with snow!

Normally the style does not wither after pollination. In a number of cases fungi have been seen on withered and even unwithered stigmas. Withering of the stigma is therefore considered to be due to fungal infection.

Germination of the pollen.

General.—The germination of the pollen has been studied by examination of killed and fixed carpels, of living material both on the plant and in culture, and of killed and fixed material from cultures. In all cases the pollen grains germinated towards the *inside* of the pollinium, causing it to split along the



Text-figures 1-3.—Sections showing germination of the Microspore. 1, The adult spore with resting nucleus and evenly vacuolate cytoplasm; × 2,115. 2, Representation of a transverse section of a pollinium, compounded from drawings of different stages of the germination of the microspore. A, Prophase and beginning of large peripheral vacuolation. B, Early metaphase. C, Beginning of anaphase. D, Late anaphase. E, Early telophase with phragmoplast beginning to cut off the generative cell against

original mother-cell wall; and through the one or more slits the pollen tubes crowd out (Plate viii, figs. 2, 5). This is in marked contrast to the pollinium of *Asclepias cornuti* where the germination of the pollen is through the "outer" wall (Gager, 1902, p. 138). In nature, the tubes emerge from the pollinium through a slit next the stigma, whose tissues they penetrate almost simultaneously (Plate viii, figs. 1, 4, and Text-fig. 5). It is a curious fact that stigmas placed among germinating pollinia in culture seemed to have no attraction for the tubes, some of which grew past the stigmatic surface without deviating from their original direction. Probably the chemotactic power of the culture medium was greater than that of the stigma.

The wall of the pollen tube is a continuation of the intine (Plate viii, figs. 2, 4, and Text-figs. 5, 6, 7). In the germinating pollen grains, on account of the great attraction the cytoplasm has for stains, it is sometimes impossible to determine whether the tube nucleus is present or not. Usually the generative cell precedes the tube nucleus into the tube (Plate viii, fig. 2; Text-figs. 5, 6, 7, 9). Occasionally the tube nucleus enters first (Text-figs. 5, left hand pollen grain, and 8). The disorganization of the tube nucleus is shown in Text-figures 5–9. The tube entirely drains the grain of cytoplasm (Plate viii, fig. 4).

The contents of the pollen tube could not be recognized in the carpel till after the embryo sac was penetrated. Between the stigma and the embryo sac the generative nucleus had divided and given rise to two slightly elliptical male nuclei of equal size which were first seen, one inside the egg cell and the other in contact with a polar nucleus. The size of the male nuclei is not much larger than that of the nucleoli of the egg and polar nuclei. The 13 chromosomes are clearly visible, probably being in the telophase condition (Text-figs. 30*a*–31). No cytoplasmic sheath was observed in connection with the male nucleus; this is not to deny the existence of one. In several instances a body was observed which is interpreted as the remains of the tube nucleus (Text-figs. 30*b*, 35*b*).

Coulter and Chamberlain (1903, pp. 135–6) point out that the time of division of the generative nucleus has no relation to the great plant groups

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the outer wall of the spore. F, Generative cell completed, large vacuoles disappearing. × 1,250. 3, Germination completed, now the pollen grain, with the generative cell free from the wall; × 2,115. In these figures note the thickening at the corners of the intine.

Text-figures 4-9.—Sections showing germination of the pollen. 4, Tube not yet formed, tube nucleus disintegrating, generative cell cut transversely showing a membrane; × 2,030. 5, One pollinium and two tubes from a second pollinium on one stigma; examination of adjacent sections showed absence of a tube nucleus from the upper left pollen grain. *ct*, cuticle. × 1,040. 6, Generative cell leading the tube nucleus into the tube; × 2,030. 7, As 6, the tube nucleus much disintegrated; × 950. 8, Generative cell in the tube behind the now formless tube nucleus; × 950. 8*a*, Diagram to show the plane of section of the germinating grain shown in Figure 8. 9, End of pollen tube showing the generative cell leading the disintegrating tube nucleus; × 950.

Text-figures 10-13.—Consecutive, slightly oblique L.Ss. through a nucellus, showing the penetration of two pollen tubes to one embryo sac. In Figure 12 the portion above the arrow had been moved away slightly during the making of the preparation. These sections include only half of the thickness of the nucellus. × 475.

Text-figure 14.—L.S., Nucellus showing distortion in the contents of the sac after entry of the pollen tube, and showing the zygote and the projecting remains of the pollen tube. This ovule is shown in Text-fig. 45. × 475.

Text-figure 15.—L.S. of tip of a nucellus (multiple epidermis) and the micropyle with the projecting remains of the pollen tube within it. The section includes the upper and lower inner faces of the micropyle. This is from the ovule shown in Text-fig. 46. × 475.

and may even be variable in a species. An example of the latter case is *Myricaria germanica* (Frisendahl, 1912, p. 30). From the few references in the literature of the Leguminosae, it seems general that the generative nucleus does not divide in the anther. This is the condition in *Albizzia lebbek* (Maheshwari, 1931, p. 246) as well as *Acacia Baileyana*. The condition of the male nuclei in the latter species suggests that the division takes place only just before the tube reaches the sac, as is thought to be the case in *Theobroma cacao* by Cheesman (1927, p. 112). The generative cell divided in the unshed pollinium of *Asclepias cornuti* (Gager, 1902, p. 137, and Finn, 1925, p. 5).

No general statement can be made as to the fate of the tube nucleus and the order of precedence into the tube. Weinstein (1926, Figs. 15, 16, 37) shows the tube nucleus leading the generative nucleus in *Phaseolus vulgaris*, and Frisendahl (1912, pp. 30, 45) says that the tube nucleus may degenerate in the grain or upper part of the style of *Myricaria germanica*.

A study of the literature referring to the male gametophyte of Angiosperms gives the impression that the formation of male *cells* is the more frequent, "cells" being taken to mean at least the definite association of a mass of cytoplasm with the male nucleus. In some cases a definite membrane has been recorded. It appears to be almost universal that before (Welsford, 1914, p. 267, for *Lilium auratum*) or soon after the liberation of the male gamete from the pollen tube the nucleus escapes from the "cell" (Guignard, 1899, p. 867, for *Lilium martagon*; Ishikawa, 1918, p. 291, for *Oenothera*), though Wylie (1923, p. 194) for *Valisneria spiralis* considers it probable that some cytoplasm enters the egg. The structure and behaviour of the male gametes of *Acacia Baileyana* will be reported on in detail in a future communication, as there are some important phenomena requiring detailed investigation in the fertilization processes of this species.

Culture.—The general method for procuring pollen germination was used successfully by Brough (1927, pp. 481–2) with *Dampiera stricta*, a plant that flowers all the year round and has a stigma with glandular hairs. He dusted the pollen on to a 5% cane-sugar solution; and germination took place rapidly, the tubes being about five times the diameter of the grain after $2\frac{3}{4}$ hours. Unsuccessful attempts were made with the pollen of *Acacia Baileyana* on the surface of cane-sugar solutions of various concentrations. Cheesman (1927, p. 111) found the best germination of the pollen of *Theobroma cacao* to take place on agar films on glass slides, the medium being 1·5–2% agar and 5% cane-sugar. Bamford and Gershoy (1930, p. 7), using 1% agar and 12% cane-sugar, were successful with the pollen of violets. In view of these successes, the agar method was used for the pollen of *Acacia Baileyana*; different concentrations of agar and sugar were tried. The successful one was 1% agar and 20% cane-sugar (cf. Plate viii, fig. 5). The ratio of sugar to agar required by these three plants is $3\frac{1}{3}$, 12 and 20 to 1 respectively. The success of the experiment requires not merely the germination of the grain (emergence of the tube), but also sustained growth of the tube. The wide difference between the conditions for sustained growth of the tube is in support of Brink's (1925, p. 161) conclusion that in Nature the conditions required for germination of pollen are less exacting than those for the growth of the tube. This is also suggested by the experiments outlined below.

For germination of the pollen of *Acacia Baileyana*, the following agars were tried as plates in petri dishes:

		Ratio $\frac{\text{Sugar}}{\text{Agar, etc.}}$
I. 2% Glucose	1% Agar, 20% Potato	0·095 : 1
II. 2% Glucose	2% Agar, 2% Peptone, ½% Pot. dihyd. Phosphate, ½% Mag. Sulphate	0·4 : 1
III. 25% Cane-sugar	5% Agar	5 : 1
IV. 12% Cane-sugar	2% Agar	6 : 1
V. 20% Cane-sugar	2% Agar	10 : 1
VI. 30% Cane-sugar	2% Agar	15 : 1
VII. 20% Cane-sugar	1% Agar	20 : 1

The lids were kept on the dishes which were left on the laboratory table (winter time). Agars V and VII were also tried as smears on slides inverted over water in a glass vessel with a loose lid. By both methods, agar VII was found to be very satisfactory; for pollinia germinated readily in contact with the medium, whether they occurred singly or in groups. Thus the medium with the greatest sugar-agar ratio, 20 : 1 (VII), gave the best results. Though fair results were given by ratios of 6 : 1 (IV) and 10 : 1 (V), the ratios 5 : 1 (III) and 15 : 1 (VI) were unsatisfactory. The failure of the media with the latter pair of ratios which are close to or within the range of those of the more successful media is probably due to a too high sugar concentration, viz., 25% (III) and 30% (VI), the highest of the series.

In good germinations the tubes attained a length of about 7 or 8 times the diameter of the pollen grain in 2½ hours after inoculation.

Pollen from flowers freshly opened on the day of inoculation never germinated readily. Pollen from the flowers of a cut shoot that had been standing in water for seven days before the inoculation still germinated well. It is concluded that the pollen is at its best from about 3 to 7 days after anthesis.

Path of the Pollen Tube.

Other than the tubes from the germinating pollinia in the tip of the style shown in Plate viii, figure 1, and Text-figure 5, the only trace of the tubes seen clearly in the style has been the cavity caused by them. This cavity is soon repaired by further growth of the style tissue. The cavity has been traced from the stigma to the ovary. This path of the tubes is through no specialized conducting tissue, and the style is so small in cross section that it is difficult to say that the path of the sixteen pollen tubes is in any particular region of it.

In the ovary the tubes pass along the floor among the thick-walled hairs which are mostly destroyed, and come into direct contact with the tips of the naked anatropous ovules (Text-fig. 22). As might be expected from the excess number of pollen tubes available (16 from one pollinium for 12 ovules) and the naked condition of the ovule, two pollen tubes are found occasionally attacking one ovule, and even entering the embryo sac (Text-figs. 10–13). These figures show that the pollen tube makes a very large wound in the tip of the ovule; but this is quickly healed and the tissue appears as vigorous as ever (Text-figs. 14, 15, and Plate viii, figs. 9, 12, 14). The pollen tube at the time of fertilization is a very delicate structure, and is easily destroyed in the making of the preparations. It is therefore very difficult to find many clear examples of it soon after fertilization when the gap in the tip of the ovule has closed up. But later on, the walls of the tube seem to harden, so that it is easily and frequently seen projecting from the tip of the nucellus in preparations made of stages up to embryos of about twelve cells in pods about 50 mm. long (length at fertilization,

0·5 mm.) (see Text-figs. 14, 15). In fact, instead of the pollen tube passing down the micropyle, the micropyle is formed round the pollen tube.

On the entry of the pollen tube there is a considerable, though variable, disturbance of the upper contents of the embryo sac (Text-figs. 13, 14), which may push the egg laterally and clear the starch grains away from above the polar nuclei. This latter would be a very useful effect in making easy the approach of the second male nucleus to the polar nuclei. On the other hand, the pollen tube may cause very little damage to the synergids (Text-fig. 33). Whatever be the disturbance, it is soon repaired and the zygote becomes attached to the distal wall of the embryo sac (Text-figs. 36, 37; Plate viii, fig. 12).

That the pollen tubes in culture ignored the presence of stigmas is in line with the findings of Martin (1913, p. 123) for the pollen of *Trifolium pratense*. He found that the stigma exerted no influence on the direction of growth of the pollen tubes, and concludes: "The behaviour of the stigma in the experiments at least indicates that its function in the germination of the pollen is to regulate the water supply; and the nature of the pollen necessitates no other function". If this is a general condition, then pollen germination in Nature must be very promiscuous, and it is chemical and physical factors such as those investigated by Brink (1925) which inhibit the growth of the foreign tubes in the stigma.

There is every indication that some at least of the excess pollen tubes of *Acacia Baileyana* enter ovules already attacked. Weinstein (1926, p. 255) describes the excess tubes of *Phaseolus vulgaris* growing to the base of the ovary and then disintegrating. The exact behaviour of the two pollen tubes on their entry to the sac has not been observed in *A. Baileyana*. In *Valisneria spiralis*, Wylie (1923, p. 195) describes them entering a synergid each.

The entry of more than one tube into one embryo sac requires a revision of the ideas of chemotaxis and that fertilization causes a reversal of chemotropism. Compton (1912*a*) describes in a *Lychnis* hybrid an ovule with two sacs which had been penetrated by two tubes right up to the sacs, and another with one sac which had been penetrated by two tubes of which one had died half-way across the parietal tissue. He makes the rather daring conclusion that these facts "seem to indicate a quantitative relation between embryo-sac and pollen tube in the matter of chemotaxis, two embryo-sacs excreting sufficient of the chemotropic substance to attract two pollen-tubes". The situation in *A. Baileyana* definitely negates this idea. There have been a number of records of more than two pollen tubes penetrating one ovule and even discharging into one embryo sac. From the following examples it would appear to be a phenomenon of the less advanced Angiosperms, though one is from the Monocotyledons. Shattuck (1905) found two tubes to one embryo sac in *Ulmus americana*; Frisendahl (1912, p. 49) says that very often more than one tube discharges into the sac of *Myricaria germanica*; Nawaschin and Finn (1913, p. 19) found in *Juglans nigra* as many as five tubes to one ovule and four discharging into one sac, three tubes arriving some time after the first tube; other cases are found in *Xyris indica* (Weinzieher, 1914), *Myosurus minimus* (Tchernoyarow, 1915) and *Oenothera* (Ishikawa, 1918, p. 295). It is significant that in *Gnetum*, where there are several eggs available for fertilization, several tubes attack the one embryo sac (Lotzy, 1899, p. 96).

FEMALE.

Germination of the Spore.

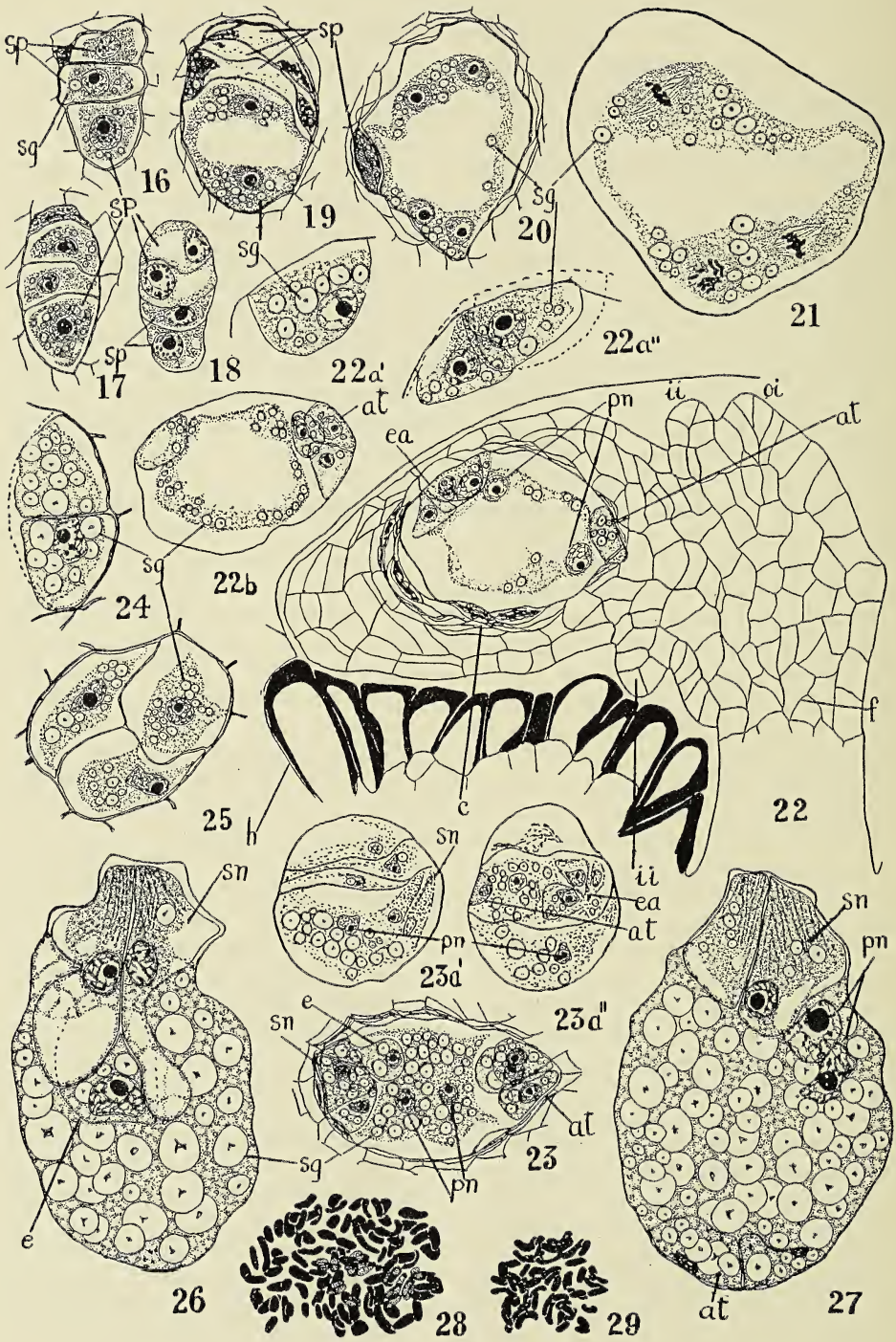
As with the microspore, the first sign of germination is the formation of vacuoles in the cytoplasm (cf. Text-fig. 16 with 17). These coalesce to form one large central vacuole about the time of the division of the nucleus, shown in Text-figure 18 and Plate viii, figure 6; the former shows two functional distal megaspores, and the latter shows a functional distal megaspore with chromosomes in the nucleus and whose central vacuole has contracted in the preparation. The nuclei resulting from the division pass to the aggregations of cytoplasm at opposite ends of the sac (Text-fig. 19). These nuclei undergo division to four (Text-fig. 20) which divide simultaneously (Text-fig. 21) to form the eight nuclei of the adult embryo sac. In the telophase of this third division, the beginning of wall formation can be discerned (Newman, 1934, Plate vii, figs. 17*a–b*, left-hand pairs of nuclei). Whether this is evanescent or is completed was not determined.

There is little evidence upon which to determine the sisterhood of the cells of the egg apparatus and upper polar nucleus. But from the direction of the spindles and from nuclear sizes and disposition in the early adult stages, it could be argued that the egg and upper polar nuclei are sisters.

Starch is formed early in the megaspores, especially in the functional ones, and is abundant in the peripheral cytoplasm, particularly in the neighbourhood of the nuclei, while the embryo sac is being completed (Text-figs. 16–21).

A. Baileyana falls clearly within Rutgers' (1923, p. 21) distinction between the formation and development (germination) of megaspores, by beginning the germination with vacuolation of the cytoplasm. The central vacuole is recorded by Saxton (1907, Figs. 6–9) for *Cassia tomentosa*, Maheshwari (1931, Figs. 22–25) for *Albizzia lebbek* and Reeves (1930*b*, p. 242) for *Medicago sativa*, in which case it becomes filled with starch from the 2-nucleate stage till the divisions are completed. This last is a similar condition to that described by Guignard (1881, p. 25) for *Acacia farnesiana*. In *A. Baileyana*, the starch develops early and remains over a long course of the development. The discussion of it will be reserved till the consideration of the situation in the definitive sac.

The separation of the polar nuclei from the distal and proximal groups of three nuclei has been observed in *Doryanthes excelsa* (Newman, 1929, p. 415), and in *Medicago sativa* (Reeves, 1930*b*, p. 242). In the former case, as in *A. Baileyana*, it was inferred that the egg and upper polar nuclei are sisters. Frisendahl (1912, p. 37, and Taf. III, fig. 87), in *Myricaria germanica*, shows the egg and upper polar nuclei being cut off from one another at the simultaneous cell formation in the egg apparatus. He also shows a definite T orientation of the spindles in his figures of the third division in the sac (p. 36), though he says (p. 37) that they have no definite orientation to one another and the long axis of the sac. Porsch (1907) says that the upper polar nucleus is sister to the egg and represents the ventral canal nucleus, the synergids being sisters. Nawaschin (1898, pp. 381–2) held that the second male nucleus fused with the sister of the egg; but he seemed to interpret the upper polar as a second egg, regarding the endosperm as a second embryo; and made comparison with the several proembryos in the sac of *Gnetum*. This question is important for the homologies of the endosperm. Schürhoff (1928), after a careful analysis of the findings of many authors, is of the opinion that neither the direction of the spindles in the last division in the embryo sac nor the orientation of the four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free micropylar nuclei are valid criteria for the determination of the sisterhood within the egg apparatus (pp. 565–6). In considering the wall formation, he could only find one significant figure in the literature, that of Frisendahl (1912, Taf. III, fig. 87) for *Myricaria germanica*; but as that shows a simultaneous cell formation, he says there is no criterion in the phragmoplast. He then considers cases of abnormal embryo sacs and the Gymnosperms, concluding that the sisterhood is synergid–egg, synergid–polar, each pair representing an archegonium, so that "double fertilization" is right in the truest sense of the word (p. 572).

Definitive Embryo Sac.

Occasionally in *Acacia Baileyana* two embryo sacs have been seen in one ovule. This is not surprising in view of the occasional occurrences of more than one mother cell or germinating megaspore in an ovule. The two embryo sacs have always been degenerate (Text-fig. 23*a*′, *a*″). The enlargement of the embryo sac destroys almost all the parietal tissue except the multiple epidermis (Text-fig. 22).

Soon after the third division in the embryo sac, the organization of cells in the egg apparatus and antipodal groups takes place, starch grains being enclosed with the nuclei (Text-figs. 22–22*b*, 24 and 25). The central vacuole becomes filled with cytoplasm containing numerous starch grains, the polar nuclei begin to approach one another and the cells of the egg apparatus to elongate (Text-fig. 23). The starch is so abundant that the spherical grains seem to be in contact at every possible point (Plate viii, fig. 8). In many cases they nearly fill the vacuolate regions of the egg and synergids and the whole of the antipodals, so that the limits of these cells are difficult to determine (Text-figs. 24, 26, 27; Plate viii, fig. 8). The starch persists in the egg during fertilization and is so packed in the sac that the polar nuclei are distorted even up to the completion of fusion with the second male nucleus (Text-figs. 30*a*–43, 58).

The enlargement of the embryo sac does not obliterate all the nucellar tissue by the time of fertilization in the Acacias as recorded by Guignard (1881, p. 28),

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Text-figures 16-20.—Development from the functional megaspore to the four-nucleate sac; × 586. 16, The functional spore proximal of three. 17, Germination beginning in the proximal spore of four. Vacuolation beginning. 18, Two distal spores germinating, note large vacuole. 19, Two-nucleate sac with central vacuole. 20, Four-nucleate sac with central vacuole.

Text-figure 21.—Embryo sac showing three spindles of the third division. 13 chromosomes visible in one of the metaphase plates. × 1,100.

Text-figures 22-22*b*.—From an ovule soon after the formation of the cells in the embryo sac. 22, L.S. of the whole ovule showing the egg apparatus, polars and part of an antipodal, also the hairs (*h*) on the floor of the ovary; × 586. 22*a*′, the egg; × 1,100. 22*a*″, the synergids (position of egg in outline); × 1,100. 22*b*, From the section adjacent to that shown in 22; part of the egg and three antipodals are shown; × 586.

Text-figure 23.—L.S., Embryo sac showing the approach to the definitive condition and the obliteration of the central vacuole; × 586.

Text-figures 23*a*′, 23*a*″.—Consecutive L.Ss. showing in the one ovule non-functional megaspores and part of two degenerating embryo sacs that had become nearly definitive; × 514.

Text-figures 24, 25.—L.S. and T.S. of the base of embryo sacs showing the antipodals as cells; × 1,100.

Text-figures 26, 27.—Consecutive L.Ss. of a definitive embryo sac. Part of two antipodals only are shown, and the upper part of the egg is in outline. × 1,100.

Text-figures 28, 29.—Metaphase plates from two abnormal endosperms. For explanation, see text in sub-section Multiple Fusion of section Fertilization. × 1,680.

and in *Albizzia lebbek* as figured by Maheshwari (1931, fig. 26). In two Papilionaceous genera there is what might be considered an advanced tendency: In *Medicago sativa* (Reeves, 1930*b*, p. 243) the sac has enlarged to obliterate all the nucellar tissue and comes in contact with the integuments except at the base; in *Trifolium* (Martin, 1914, p. 164) the sac becomes long by resorption of the chalaza. In *Trifolium* the central vacuolation remains; in *Vicia* and *Medicago* it becomes filled with cytoplasm (loc. cit.).

Starch.—The starch grains vary considerably, from the size of the smaller nucleoli of the sac to one-quarter of the width of the sac. In permanent preparations the grains appear under the microscope as spherical refringent bodies with the hylum as a black or bright area according to the focus (see in Plate viii, fig. 8). The starch does not seem to be of a typical nature, for though staining blue with iodine, it could not be removed from the sac in sections of fixed material by the usual means, such as prolonged hydrolysis with dilute sulphuric and hydrochloric acids, diastase, saliva, and acetone followed by saliva. If in any case the starch was removed, so were the whole sections that had been fixed to the slide with egg albumen. These tests were made on sections of both *Acacia Baileyana* and *A. discolor*. Before fertilization the starch takes no stain with Haidenhain's Iron Alum Haematoxylin or with Safranin. After fertilization it takes a purplish stain with the Haematoxylin and a dull red stain with Safranin.

The presence of starch in the embryo sac seems to be of wide and variable occurrence; and frequently the form of it is not normal. Cheesman (1927, p. 108) refers to starch grains, "if the term is used in its wide sense", in the embryo sac of *Theobroma cacao*; and describes how they do not take stains before fertilization, but do so afterwards, as has been described for *A. Baileyana*. Dahlgren (1927), reviewing the records up to about 1927 of the appearance of starch in the embryo sac, gives lists where, as here, the starch obscures the structures of the sac (p. 374), and appears in the egg apparatus and antipodals (p. 375). Is it possible that the centrosomes figured by some authors as occurring in the egg are small starch grains, as for instance, by Schaffner (1897) in *Sagittaria variabilis*? Dahlgren (1927) lists the first appearance of starch in different species at times ranging from the megaspore mother cell stage to the fusion of the polar nuclei (p. 375), and says that it reaches its maximum just before fertilization, and then is more or less rapidly consumed (p. 376). Its occurrence may even vary within a species (p. 374).

Starch in the embryo sac seems to be of wide occurrence in the Leguminosae. Guignard (1881, p. 25) records it in the functional megaspore of *Acacia farnesiana*, as it is here in *A. Baileyana*; Maheshwari (1931, p. 248) never finds it before the 8-nucleate stage in *Albizzia lebbek* where it persists, as here, even to the stage of the free-nucleate endosperm. Among the Papilionaceae it is found by Reeves (1930*b*, pp. 242–3) from the stage of the two-nucleate sac to maturity in the neighbourhood of the egg apparatus and polar nuclei in *Medicago sativa*; by Martin (1914, p. 164) it is described in the micropylar end of the nucellus only of *Trifolium* and *Vicia*, and filling the sac in *Medicago*; in *Arachis hypogea*, it fills the sac before fertilization (Reed, 1924, p. 380). Dahlgren (1927, p. 380) lists starch grains in the embryo sacs of species from five families of the Rosales other than the Leguminosae and Rosaceae, there being only one species of the latter—*Spiraea lindleyana* in which Péchoutre (1902, p. 92) describes the sac filled with starch before fertilization.

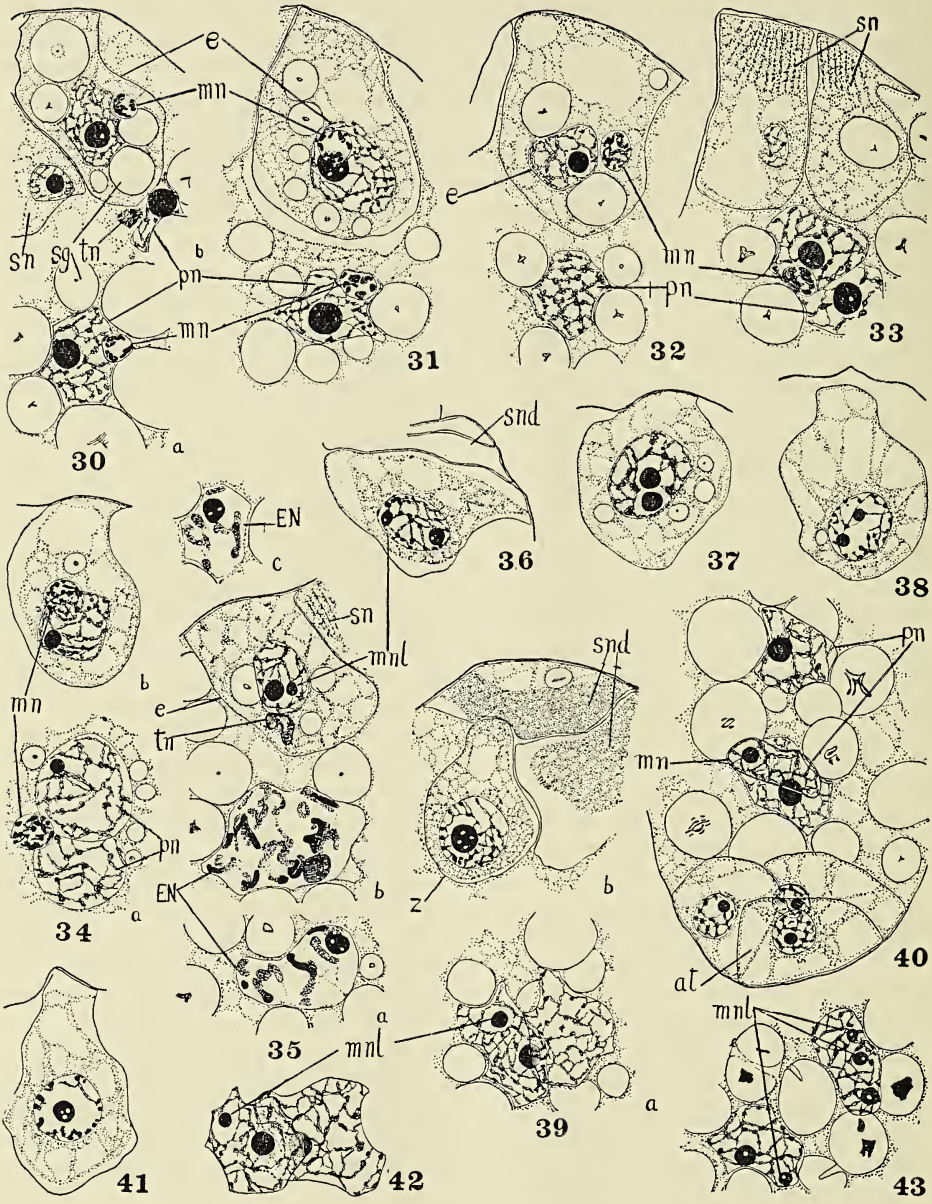
Juel (1911, p. 9), examining *Hippuris vulgaris*, considers starch formation in the embryo sac to be a consequence of delayed fertilization, a possibility concluded by Maheshwari (1931, p. 249) from observations on *Albizzia lebbek*. But in *Verbena*, Kanda (1920, p. 63) describes the starch as occurring "not only a little before fertilization, but more especially after fertilization has occurred", a condition that is contradictory of the conclusion just mentioned. And in such cases as *Acacia Baileyana* and others mentioned above where starch appears in the early stages of germination of the megaspore, it cannot be due to delayed fertilization.

Egg Apparatus.—Though variable in their arrangement, the cells of the egg apparatus are not unusual in form. The synergids are largely vacuolated at the base, with a dense mass of striated cytoplasm in the upper part and the nucleus at the junction of the two zones, while just below the dense cytoplasm there is a hook or notch in their contour, which does not appear to be due to plasmolysis (Text-figs. 26, 27, 33, and Plate viii, fig. 8). The egg, as usual, has the nucleus in the lower part and large vacuoles in the upper part (Text-figs. 23, 26, 31, 32).

The variations of arrangement are in the position of the egg, which may be below or beside the synergids. In case it is below them their great length causes the egg to be half-way down the sac, sometimes even below the polar nuclei (Text-figs. 26, 27). A case in which it was level with the synergids is shown in Text-figures 32 and 33, which are consecutive sections. Cases where the egg had been below the synergids before fertilization are shown in Text-figures 36 and 39*b*. The egg is considered to be drawn up to the top of the sac after fertilization by adhesion to the synergids that are being resorbed. When the egg is level with the synergids it is attached to the embryo sac wall at the top or just below the top (Text-figs. 23, 32, 33, 58, 59; Plate viii, fig. 9).

The egg apparatus as described above is generally in line with that described by Guignard (1881, p. 28) for several species of *Acacia*. Weinstein (1926, p. 254) recorded the disappearance of the synergids before fertilization in *Phaseolus vulgaris*. Saxton (1907) makes no reference to cell formation in the egg apparatus of *Cassia tomentosa*, and figures only nuclei (Fig. 10). Dahlgren (1928) has reviewed the records of the presence of a hook or notch on the synergid. In a list of plants in which it had been recorded up to about 1928 (pp. 434–7) he mentions no Leguminosae. *Acacia Baileyana* must now be added to the list. Of the Rosales, he only mentions those recorded by Pace (1912), namely: *Parnassia palustris*, *Saxifraga cordifolia*, *Heuchera brixoides* and *Drosera rotundifolia*; and by Himmelbaur (1911, fig. 19), *Ribes pallidum*. He concludes that the notch is generally a true structure and not due to plasmolysis (pp. 437–8), arising through the mechanics of development ("dürfte ihr Entstehen allein von entwicklungs-mechanischen Ursachen bedingt sein"), that it may or may not appear in one plant, and that certain families more than others are liable to manifest the phenomenon (p. 441).

Polar Nuclei.—The antipodal polar nucleus migrates towards the egg near which it and the upper polar nucleus remain associated (Text-figs. 23, 26, 27; Plate viii, fig. 8). They are so distorted by the starch grains that it is impossible to determine whether they differ in size. Their limits are so obscured by the starch grains that sometimes the only clear indications of them are the two large nucleoli (Plate viii, fig. 8). Their distortion and unfused condition up to and during fertilization can be seen in Text-figures 26, 27, 32, 33, 40 and 42.



Text-figures 30*a*-43.—Stages of fertilization; × 1,300. 30*a*, *b*, In the one embryo sac, the male nuclei against a polar and the egg nucleus respectively. Chromosomes definite (13). 31, Male nuclei enlarging against the egg and polar nuclei, chromosomes still definite (13). 32, 33 (consecutive sections), Male nuclei with chromosomes losing definition, against the egg and polar nuclei. 34*a*, *b*, Male nuclei approaching the resting condition against the polar nuclei and egg nucleus. 35*a*, *b*, *c*, Three consecutive sections showing the primary endosperm nucleus with three nucleoli and about thirty-nine spongy prophase chromosomes, while the zygote shows a large and a small nucleolus in the resting nucleus. The tube nucleus, *tn*, is outside the zygote. 36-38, 41, The zygote,

In *Lilium martagon*, Guignard (1899, p. 865) described the upper polar nucleus to be larger than the egg nucleus, as is recorded here; but it could not be determined whether *Acacia Baileyana* agrees in the larger size of the lower polar nucleus. The time of fusion of the polar nuclei seems to be indefinitely distributed among the Leguminosae. In the Papilionaceae, fusion is either (1) before fertilization, (2) during fertilization, or (3) variable in time. Records of (1) are a statement of its general occurrence by Guignard (1881, p. 142) and for *Crotalaria sagittalis* by Cook (1924, p. 440); of (2) are a statement of its occurrence in some Vicieae by Guignard (loc. cit.) and in five species from among *Trifolium*, *Vicia*, and *Medicago* by Martin (1914, p. 163); of (3) are statements of its occurrence in *Phaseolus vulgaris* by Weinstein (1926, pp. 254–5) and in *Medicago sativa* by Reeves (1930*b*, p. 243). In the Caesalpineae, Guignard (1881, p. 142) says polar fusion is before fertilization; confirmed by Saxton (1907, p. 4) for *Cassia tomentosa*. In the Mimoseae, Guignard (1881, pp. 28 and 142) describes the fusion before fertilization, but in *Albizzia lebbek* (Maheshwari, 1931, p. 248) and *Acacia Baileyana* it is not before fertilization. Thus the rarer occurrence, the late fusion, seems to be shared by some Mimoseae and some Papilionaceae. In *Myricaria germanica*, Frisendahl (1912, p. 54) records the fusion of the polar nuclei very late, after the division of the zygote, and connects it with the reduced endosperm formation. There is a suggestion in this that the late fusion is an advanced condition. But in some Australian plants recently examined, the more advanced types show fusion before fertilization (*Styphelia longifolia* and *Dampiera stricta*—Brough, 1924, p. 171 and 1927, p. 487; *Doryanthes excelsa*—Newman, 1929, p. 416), and the less advanced during fertilization (*Grevillea robusta*—Brough, 1933, p. 55; and *Acacia Baileyana*).

Antipodals.—The antipodals are cells (Text-figs. 24, 25) and are still strongly organized at the time of the formation of the primary endosperm nucleus (Text-fig. 40), but disintegrate soon afterwards.

Guignard (1881, p. 141) describes the antipodals in the Papilionaceae as generally having a delicate membrane and degenerating before fertilization, confirmed by the work of Martin (1914), Cook (1924, p. 441), and Weinstein (1926, p. 254); while in the Caesalpineae and Mimoseae they are more robust and persist till about or after fertilization as described by Saxton (1907, p. 3) for *Cassia tomentosa* and by Maheshwari (1931, p. 248) for *Albizzia lebbek* respectively, and above for *Acacia Baileyana*. The last two sub-orders possess the primitive feature in common, as would be expected. *Grevillea robusta*, an Australian Protead, also has persistent antipodals (Brough, 1933, p. 55).

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showing the nucleus passing from the resting condition to just before the organization of the prophase chromosomes, and the two unequal nucleoli becoming equal and finally appearing to have fused. 39*a*, The two polar nuclei after the second male nucleus has fused with one of them. The small male nucleolus is in the polar nucleus lying below the second polar nucleus, whose nucleolus is not shown. 39*b*, From the same sac as 39*a*, showing the zygote with the nucleus having passed back from prophase to the resting condition. 40, Base of sac, earlier than 39*a*, *b*, showing the enlarged resting male nucleus lying against one polar. Note the different sizes of the nucleoli; also the antipodal cells. 41, See above. 42, The two polar nuclei after the fusion of the male nucleus with one of them. One polar nucleus, with the small male nucleolus in it, is lying above the other polar nucleus. 43, Two polar nuclei containing one and two small nucleoli representing probably three male nuclei.

In all these figures note the starch which disappears from the later zygotes, and in the sac finally shows signs of corrosion (43).

Fertilization.

The Nuclei at the Time of Contact.

Both male nuclei at the time of contact show 13 deeply-staining chromosomes. At the same time the egg and polar nuclei have coarsely reticulate chromatin with a large nucleolus (Text-figs. 30*a*–31). The shape of the male nuclei is very slightly elongated with occasionally a slight curve, or they may be sometimes apparently spherical.

It seems general among the Angiosperms that when the male gamete discharged into the sac is a cell with or without a membrane, it is finally the naked nucleus that makes contact with the egg or polar nucleus. In *Valisneria spiralis*, however, Wylie (1923, p. 196) describes a cell in contact with the egg and says that some or all of the male cytoplasm enters the egg.

The male nucleus of *Acacia Baileyana* is definitely not vermiform, being only very slightly elongated if not spherical. In a recent text-book of Plant Cytology, Guilliermond, Mangenot and Plantefol (1933, p. 788) say that the male nucleus is as a rule ("suivant la règle générale") vermiform and spiral. From the literature available to me certain considerations arise that may modify this statement. The following are records of vermiform male nuclei: *Lilium martagon* and *Fritillaria tenella* (Nawaschin, 1898, p. 378), *Lilium martagon* (Guignard, 1899, p. 867), *Caltha palustris* (Thomas, 1900*a*, p. 318), *Lilium martagon* and *L. auratum* (Blackman and Welsford, 1913, p. 112, and Welsford, 1914, p. 267), *Trillium grandiflorum* and *Lilium martagon* (Nothangel, 1918, pp. 147–9) and *Triticum durum hordeiforme* Hort. var. *cubanka* (Sax, 1918, p. 315). These plants belong to the Monocotyledons or Ranales. The following are records of elliptical or spherical male nuclei and the plants belong to the Dicotyledons other than the Ranales: *Myricaria germanica* (Frisendahl, 1912, p. 52), *Asclepias cornuti* (Finn, 1925, p. 19), *Phaseolus vulgaris* (Weinstein, 1926, p. 255), *Theobroma cacao* (Cheesman, 1927, fig. 10), *Viola odorata* var. *praecox* (Madge, 1929, p. 567), *Grevillea robusta* (Brough, 1933, Text-fig. 69), and now *Acacia Baileyana*. This apparent difference of distribution must be regarded with caution as the numbers are small. It may also be noted that the vermiform nuclei are described in the earlier records.

From the literature quoted in the last paragraph with the addition of a further paper on *Caltha palustris* (Thomas, 1900*b*, p. 531), it appears that *Acacia Baileyana* follows the general rule in the greater increase in size of the male nucleus that fuses with the polars. This is probably to be correlated with the longer period during which it is free in the embryo sac. The male nuclei usually attain a spherical form before or after contact, but this is prevented for the enlarging second male nucleus in *Acacia Baileyana* by the starch grains.

Cases recorded other than that of *Acacia Baileyana* where the male nuclei on contact are not in a typical resting condition are *Fritillaria pudica* (Sax, 1918, p. 313), with a dark staining network; *Myricaria germanica* (Frisendahl, 1912, p. 52) with a kind of telophase condition not showing definite chromosomes; and *Lilium martagon* and *L. auratum* (Welsford, 1914, p. 268) with a spireme. The complete resting condition has been described in many plants, for records of which reference should be made to the literature quoted in the two preceding paragraphs with the addition of *Cypripedium* (Pace, 1907, p. 359), *Oenothera* (Ishikawa, 1918, p. 294) and *Valisneria spiralis* (Wylie, 1923, p. 196 and Fig. 8). The resting condition at the time of contact of the male nuclei is characteristic of the

Coniferales (Guilliermond, etc., 1933, pp. 784–5) and of *Bowenia* and some other Cycadales (Lawson, 1926, p. 377). Thus the distribution among the Phanerogams of the condition of the male chromatin at the time of contact appears to be indefinite.

The Course of Fusion.

The earliest stage of fertilization seen was when one male nucleus was within the egg cell and the other was closely adjacent to one of the polar nuclei (Text-figs. 30*a*–31). There does not seem to be any uniform direction of approach of the male nucleus to the egg nucleus.

The chromatin changes are similar in both male nuclei during fertilization. Fusion with the egg takes place in advance of that with the polar nucleus, though the times are variable. In triple fusion, about the middle of the sac, the second male nucleus fuses with one polar nucleus, the product then fusing with the other polar nucleus. The chromatin of the egg and polar nuclei undergoes no apparent change during fertilization.

As fusion approaches, the male nuclei enlarge, their chromosomes become less definitely organized (Text-figs. 31–34*b*), and probably in both cases they attain the resting condition with a nucleolus and coarse reticulum as observed in the second male nucleus (Text-fig. 40). With the disappearance of the male nucleus from outside the egg and polar nuclei, the latter two each acquire an extra nucleolus which is small and of similar size to that of the resting male nucleus seen in contact with the polar nucleus (Text-figs. 36, 39*a*, 42). The fusion therefore takes place in the resting condition, the male nuclei having just passed through telophase. In the zygote, the nucleoli become equal in size and ultimately fuse (Text-figs. 37, 38, 39*b*, 41). About the time of fusion of the nucleoli the chromatin of the zygote appears to enter on a spireme stage preparatory for division (Text-figs. 38, 41), but as chromosomes are about to be organized at the periphery, the nucleus returns to the resting condition which it maintains for a long time (Text-figs. 39*b*, 59). In the polar position there are visible normally the two large nucleoli representing the polar nuclei and one small nucleolus representing the male nucleus (Text-figs. 39*a* and 42). Only one example of the primary endosperm nucleus was seen, and that was in prophase of division with about 39 rather spongy chromosomes and three nucleoli (Text-figs. 35*a-c*). It seems that the polar and male nucleoli may not fuse on account of the rapid onset of division of the primary endosperm nucleus.

As well as the positive evidence of fertilization and triple fusion given above, there is the indirect evidence of chromosome number. The number of chromosomes found in the last division in the embryo sac (and therefore in the egg) and in the sperm nucleus is half that found in various sporophytic tissues (Newman, 1934). The number of chromosomes found in the sperm and polar nuclei, similarly, is one-third the number found in the division of the primary endosperm nucleus and of its two daughter nuclei (loc. cit.).

There seems to be no definite distribution among the Angiosperms of the chromatin changes during fertilization. In general, the nuclei taking part may be in the resting condition or prophase at the actual time of fusion (Guilliermond, etc., 1933, pp. 788–9). If the male nuclei undergo change of phase after they enter the sac, obviously the nucleus that fuses later will have proceeded further in its change than the other one. For instance, here in *Acacia Baileyana*, the male

nucleus that fuses with the polar nucleus proceeds further into the resting condition than that fusing with the egg; and in *Triticum durum hordeiforme* in the triple fusion the second male nucleus (also the polar nuclei) passes on to the metaphase before fusing, while the first male nucleus and the egg nucleus fuse earlier, in the spireme condition (Sax, 1918, pp. 316–7). In *Fritillaria pudica* the male nuclei usually pass into the resting condition for fusion, but sometimes they pass beyond that into a spireme (op. cit., pp. 313–5). Fusion with the male nuclei at the very end of telophase, as in *Acacia Baileyana*, is found in *Myricaria germanica* by Frisendahl (1912, p. 49). In *Phaseolus vulgaris*, Weinstein (1926, p. 255) has described all the nuclei in the resting condition at the time of fusion.

The relative times of the two fertilizations and the order of the triple fusion in *Acacia Baileyana* present no unusual features (see Guilliermond, etc., 1933, pp. 788–9), the order of the triple fusion (male with one polar, then with the other polar) being of a less general type.

In view of a later discussion, attention is called to the appearance of the nucleoli in the fusion nuclei, whether endosperm or zygote. As described above, after fusion there is to be seen a smaller additional nucleolus in the fusion nucleus. Cheesman (1927, p. 114) in *Theobroma cacao* has described two and three nucleoli in the fertilized egg and polar nuclei respectively. The smaller size of the additional nucleolus is recorded in both cases for *Oenothera* by Ishikawa (1918, p. 294) and in the primary endosperm nucleus of *Viola odorata* var. *praecox* by Madge (1929, p. 570).

MULTIPLE FUSION (POLYSPERMY).

Direct Evidence.

Several cases were observed where at the polar position there were two nuclei (after fertilization of the egg) that together had more than three nucleoli between them—mostly 5 nucleoli (Text-fig. 43). This would not attract much attention, but for the fact that two of them (not in the one nucleus) were always much larger than the others and of a size similar to that of the nucleoli of the polar nuclei before fertilization, and that the others were of a size similar to that of the additional nucleolus in the zygote and of the nucleolus in the resting male nucleus (Text-fig. 43; cf. Text-figs. 35*b*, 36, 40, 42). The immediate inference is that these nucleoli are evidence of additional sperms. The presence of the starch grains made it impossible to observe whether any of these small nucleoli were in separate nuclei. There were two possible cases of four sperms in the sac at the time of contact with egg and polar nuclei. Other direct evidence, that is to say, phenomena that are capable of being in the line of causation (not results), is given below.

Passing backwards in time: Two pollen tubes have been seen to penetrate to one embryo sac in several cases, though the difficulties of preparation have prevented a correlation of this occurrence with the presence of extra nucleoli at the polar position. The naked ovule is a suitable object for the attack of more than one pollen tube. One fully germinated pollinium (the unit of pollination) provides 16 tubes for the 12 ovules in the carpel; so that there are 4 surplus pollen tubes. Two pollinia have been seen germinated on one stigma in several cases, whence the 32 pollen tubes would provide two for each ovule and eight surplus. In the one case where three pollinia were seen on one stigma there would be four tubes for each ovule.

In view of the foregoing, one would be surprised, not at the occurrence of polyspermy, but at its absence. There was never any suggestion in the preparations of additional male nuclei associated with the egg.

Indirect Evidence.

As polyspermy is only suggested in connection with endosperm formation, the phenomena considered here can be little more than chromosome numbers. The normal number of chromosomes in the endosperm is 39; and in most endosperms none of the division figures seen suggested any deviation from this number. Two aberrant endosperms were found.

In one endosperm the division figures showed an enormous number of chromosomes and the metaphase plates frequently appeared branched in side view (Plate viii, fig. 7). The examination of such a plate in polar view is complicated by the presence of the vertical arm, on which the chromosomes would appear as paired halves. Text-figure 28 shows a plate of this kind. Allowing for the vertical arm, there are considered to be approximately 104 chromosomes represented; but if certain paired structures are pairs of half chromosomes at the inclined edges of the plate (cf. Plate viii, fig. 7), the number is reduced to approximately 91. These numbers are 8ϕ and 7ϕ respectively, and would be attained if 6 or 5 sperms took part in endosperm formation. All plates in this endosperm appeared of similar size.

In another endosperm, the chromosome plates of the division figures show definite differencees of number. One metaphase was observed in polar view to have 52 (4ϕ) chromosomes (Text-fig. 29), a nucleus in prophase was estimated to have 13 (ϕ) chromosomes, and another in anaphase had 13 (ϕ) chromosomes in each group. Such a condition could arise if three sperms fused with one polar nucleus and the second polar nucleus divided without fusion, or if two sperms and two polar nuclei fused while the third sperm divided without fusion.

It may be objected that it is well known that nuclear fusions and abnormalities of nuclear division are often found in endosperm tissues. But it is difficult to derive the numbers found here from nuclear fusions or failures of cell division (some of the latter have been observed) based on the normal endosperm number of 39. In view of evidence of polyspermy in other plants, a case has been made out for further investigation.

Discussion of Polyspermy.

Even if the above interpretation of polyspermy in endosperm formation be not proved by further work, it will have served a purpose in directing attention to the presence of extra sperms in the embryo sac of Angiosperms; for in three recent text-books of a morphological-cytological nature by Sharp (1921), Schürhoff (1926) and Guilliermond, Mangenot and Plantefol (1933) there is only slight reference to polyspermy, and then it is considered only in association with embryo formation. This association is the only one entertained in the literature that has come under my notice.

It may be objected that the phenomenon that drew attention to the possibility of polyspermy—the extra nucleoli after polar fusion—is not a valid criterion. This objection was made by Ernst (1902) when describing double fertilization in *Paris quadrifolia* and *Trillium grandiflorum*; Nothangel (1918, p. 150), in *Lilium martagon* and *T. grandiflorum*, described the male nucleus without a nucleolus before fusion and the zygote nucleus with various nucleoli after the

fusion. Sax (1918, p. 313) describes the zygote of *Fritillaria pudica* as having several extra nucleoli. On the other hand, Campbell (1911, p. 783) describes the polar fusion nucleus of *Pandanus coronatus* as showing the nucleoli of the component nuclei—2–6 nuclei being concerned. Doubtless, by itself, increase in the number of nucleoli would be of little value for determining polyspermy, but when the increase appears as an occasional feature in a definite manner, and is associated with other phenomena pointing to the same possibility, it must be allowed to bear some weight.

It is not unreasonable to suggest a multiple fusion of nuclei available in the embryo sac, for there is the example of *Pandanus coronatus* quoted above, and the multiple fusions in the formation of the primary endosperm nucleus of *Peperomia pellucida* (Johnson, 1900). Weinstein (1926, p. 255) has described a possible case of the fusion of a tube nucleus and a male nucleus with a polar nucleus in *Phaseolus vulgaris*. In reference to the fact that sometimes in *A. Baileyana* two primary endosperm nuclei of different chromosome constitution may be formed from amongst the polars and sperms, it is to be noted that Campbell (1911, p. 783) has described two primary endosperm nuclei in *Pandanus coronatus*, and Frisendahl (1912, p. 50) suggests that in *Myricaria germanica*, though only one sperm can fuse with one polar, both polars might fuse with a sperm each.

There are a number of records of extra sperms in the embryo sac, but attention seems only to have been directed to the possibility of polyembryony or polyploidy arising therefrom. From this point of view, Schürhoff (1926, pp. 312–3) discusses the dispermy recorded in *Gagea lutea* by Němec (1912, p. 173) and in *Saxifraga granulata* by Juel (1907, p. 18), and though he records Derschau's (1918, p. 262) observation of dispermy with an antipodal in *Nigella arvensis*, he does not consider any case of it in connection with endosperm formation (pp. 338 et seq.). Ishikawa (1918, p. 295) describes extra sperms in the embryo sac of *Oenothera*, but neither he nor Gates (1928, p. 428) who discusses it considers the possibility of polyspermy with the polar nuclei. As many as six sperms have been found to enter the embryo sac of *Myricaria germanica* by Frisendahl (1912, p. 49) and of *Juglans nigra* by Nawaschin and Finn (1913, p. 19).

The formation of endosperms with more or less than 3ϕ chromosomes is not unknown. Renner (1914) and Ishikawa (1918, p. 295) record 2ϕ endosperms in *Oenothera* where there is only one polar nucleus. Sax (1918, p. 313) describes in *Fritillaria pudica* the disintegration of one of the chalazal nuclei at the 4-nucleate stage and the doubling of the chromosomes of the other at the third division, wherefore the endosperm has 4ϕ chromosomes. The suggestion that one of the extra male nuclei may have divided without fusion in *Acacia Baileyana* is not without a parallel in that Chamberlain (1916, p. 364) records the division of extra sperms in two cases in the Cycad *Stangeria paradoxa*.

By some authors the endosperm is thought to be a degenerate embryo. Sargant (1900, p. 708) suggests that the third nucleus in the triple fusion secures the degeneracy of the resulting tissue. But in *Acacia Baileyana*, the endosperms appearing polyploid do not seem any more "degenerate" than normal ones. Campbell (1902, pp. 785–6) considers that the fusion of the second male nucleus with the only available nucleus in the sac is not surprising and must be regarded as more or less accidental. The case of *Peperomia* is for Coulter

(1911, p. 383) "positive evidence that in the embryo sac there is some condition that favours nuclear fusions, quite apart from what may be called sex attraction", and he considers that "the product of such fusions . . . is simply *growth* and not *organisation*" (page 384). He concludes that triple fusion, etc., is not necessarily of phylogenetical significance, but rather a physiological problem of the conditions in the embryo sac favourable to miscellaneous nuclear fusions. Polyspermy in connection with endosperm formation, as suggested for *Acacia Baileyana*, is therefore a phenomenon reasonably to be expected.

Seed Production.

CHANGES IN THE CARPEL.

At the time of fertilization, the carpel is not more than 0·5 mm. long and, if all the ovules develop to seeds, it is about 100 mm. long at the time of dehiscence. The outward sign of post-fertilization change is that the carpel turns pink, then bright crimson, which slowly becomes duller till the full-sized pod turns green. The seeds are equally spaced in the mature pod, showing that in the young carpel they are not in pairs, as might be thought from transverse sections, but are really alternating. The breadth and length of the carpel increase for a time, and then length alone till the pod is about 25 mm. long, when the breadth continues to increase with the length. The final breadth is about 12 mm. The seeds whose funiculus has increased to 5 mm. in length stand along the middle line of the pod with their long axis parallel thereto. The significant histological changes and the dehiscence mechanism have been described in a previous paper (Newman, 1934).

CHANGES IN THE OVULE TISSUES.

The Receptacle (Nucellus).

At the time of fertilization, the embryo sac has destroyed practically all the parietal tissue above and the receptacle tissue to the side. Little more than the multiple epidermis above, and one or two layers at the side, is left. This condition persists for some time (Text-figs. 13, 14; Plate viii, figs. 9, 12, 14). In the older of these stages it is probable that some of the layers are of secondary growth replacing loss by resorption. The endosperm-filled sac is enclosed on the upper part by epidermal tissue only, which is multiple at the top (Plate viii, fig. 16), the chalazal tissue, though it becomes massive by secondary growth (Plate viii, figs. 13, 14, 16, 17), is finally overtaken by the growth of the sac (Plate viii, fig. 18). The antipodal pocket, bordered by thick-walled cells, persists into the mature seed and at some stages is overarched by the endosperm (Plate viii, figs. 9, 13). There is a column of elongated cells below the antipodal pocket connecting the vascular bundle of the ovule with the embryo sac (Plate viii, figs. 13, 16).

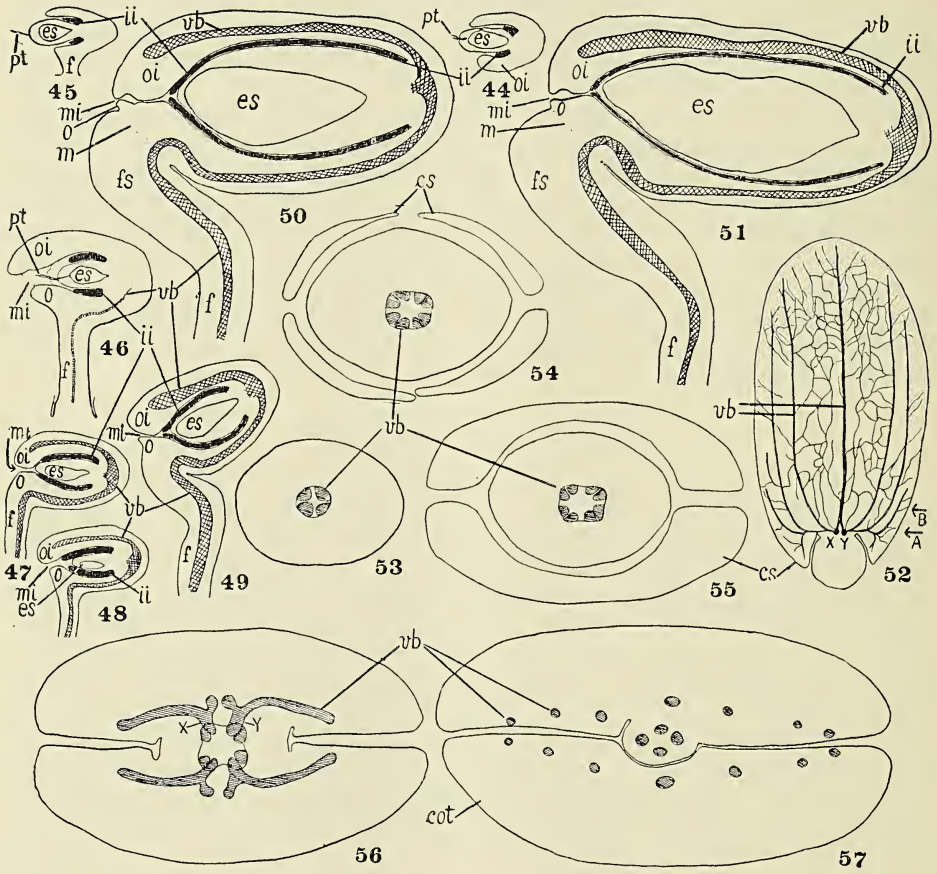
Guignard (1881, p. 29) describes the young embryo of the Acacias as in contact with the multiple epidermis of the nucellus. In two other Australian Dicotyledons with a massive integument, the embryo sac crushes all (*Aegiceras majus*, Carey and Fraser, 1932, p. 344), or almost all (*Grevillea robusta*, Brough, 1933, p. 61), of the nucellus after fertilization.

The Integuments and Funiculus.

One of the first microscopic changes after fertilization is the development of the integuments, so that the ovule, which was completely naked, acquires fully

developed integuments, forming a micropyle by the time of the first division of the zygote. The inner integument is epidermal in origin and its primordium is the first to appear (Text-fig. 22). The outer integument is subepidermal in origin. When growth finally begins, the outer integument exceeds the inner, closing over the nucellus before the inner is level with the top of the embryo sac (Text-fig. 46). The inner integument finally forms the lower part of the micropyle that is formed round the projecting remains of the pollen tube.

The inner integument is a simple urn-shaped upgrowth from a ring, two cells broad, around the chalazal epidermis. The region of the chalaza from



Text-figures 44-51.—L.Ss. showing the development of the seed after fertilization. All, except 44, are parallel to the plane of the funiculus, 44 being perpendicular to that plane. The inner integument is shown dead black, the vascular bundle is cross-hatched. The remains of the pollen tube are seen in 44-46. 44-46, × 60; 47-51, × 30.

Text-figure 52.—Cotyledon showing the vasculation on the inner face. X and Y, the two bundles leading from the embryo axis (cf. 56). The arrows A and B indicate the planes of section shown in 56 and 57; × 7.

Text-figures 53-57.—A series of transverse sections of an embryo at different levels from the radicle up to the base of the cotyledons. 53 is just below the limit of the cotyledon spurs. X and Y correspond to X and Y in 52. 56 and 57 are at the level of A and B in 52. × 25.

which the outer integument develops can be regarded as a ring containing a gap into which is fitted the funiculus. The final form of the outer integument is that of an urn with a gap down one side into which is fitted the funiculus. As this "urn" is developed by upgrowth of the broken ring primordium, whose "broken ends" are in organic meristematic connection with the funiculus, it appears to pull the funiculus parallel with the long axis of the growing seed (Text-figs. 45–47). The use of the word "pull" is doubtless inaccurate, but it describes the appearance. The real cause is probably differential growth of tissues in the funiculus, producing curvature. The general development of the integuments and funiculus is shown in Text-figures 44–51. There is a second bend in the funiculus, level with the top of the nucellus, which brings it back on itself along the surface of the seed for some distance. From there it is turned at right angles towards its insertion on the carpel (Text-figs. 49–51). From the second bend in the funiculus there is an outgrowth (*o*) to complete the micropyle (Text-figs. 46–51).

There is great difference between the integuments in size. The inner is only two cells thick, except at the micropyle of seeds well advanced (Plate viii, figs. 9, 12, 14, 16). Its inner layer is the more robust and survives being crushed by the growth of the contents of the embryo sac (*i.i.l.* in Plate viii, figs. 16, 17). The outer integument is massive and has a single vascular bundle which, with the vascular bundle of the funiculus, lies in the broad plane of the seed (Text-fig. 51; Plate viii, figs. 16, 19). The micropyle can be identified in the full-grown seed (cf. Plate viii, fig. 16, of half-grown seeds).

On the free part of the funiculus, parallel with the seed, growth in thickness occurs so that a considerable swelling takes place, mostly on the side away from the seed. This results in a cushion of pithy tissue lying against the side at the micropylar end of the seed (Text-fig. 51; Plate viii, figs. 19, 20). This tissue contains chlorophyll and does not shrivel away when the seed dries. It is usually referred to as an "aril". Ontogenetically it is a swelling of the funiculus, semicircular in transverse section and situated some distance from the insertion of the funiculus on the chalaza.

In their order of origin and in their form and relative rate of growth, the integuments are very similar to those of most of the other Leguminosae already described; but they differ in the remarkably late time of their development, there being in the literature no suggestion that the integuments do not continue to grow immediately after their initiation. Normally, integuments are completed by the time the embryo sac reaches maturity (Martin, 1914; Weinstein, 1926, p. 252; Reeves, 1930*b*; Maheshwari, 1931; also Péchoutre, 1902, p. 148, for the Rosaceae). Guignard (1881, p. 27) describes the integuments in *Acacia* as being level with the top of the nucellus when the embryo sac is full size. It is possible that he examined carpels not long after fertilization. He also describes the micropyle as displaced towards the funiculus (p. 28), but in his figure (fig. 11) it is not so far displaced as it is in the outer (only) integument of *Cassia tomentosa* described by Saxton (1907, p. 2). At the same time Saxton describes the extraordinary phenomenon of the outer integument being developed to the full on the funicular side of the nucellus. Pammel (1899, p. 216) relates the seeds of *Acacia* to those of *Desmanthus* (Eumimoseae) and *Cassia* (Caesalpineae) on account of the inner integument having been destroyed. He is probably neglecting the micropylar region which we have seen to persist in *Acacia*

Baileyana. Allowing for this fact, it would still appear that *Acacia* and *Desmanthus* (Mimoseae) are in this more like the Papilionaceae than the Caesalpineae in which the inner integument may have more than four layers of cells (Pammel, 1899, p. 117).

Guignard (1881, p. 28) describes a vascular bundle in the outer integument of Acacias as in the species here described. Speaking of the vasculation of the ovule, Le Monnier (1872, p. 279) says: "Nous voyons donc la nervation tendre vers une forme réduite, déjà signalée et désignée sous le nom de nervation en *bouche* complète". He says that this simple vasculation is in most Caesalpineaceae and Mimosaceae (*Albizzia, Mimosa, Acacia*), and instances Papilionaceae with complex vasculation.

The funicular swelling or aril may be regarded as a third integument that arises late. Among the Dilleniaceae (Gilg, 1895, p. 107), Leguminosae (Taubert, 1894, p. 95) and Passifloraceae (Harms, 1895, p. 77) there is every gradation from less than the condition described here to complete envelopment of the seed, even of anatropous seeds. The lateness of development of the first two integuments in *Acacia Baileyana* shows that lateness of development is no bar to integumental status.

General.

Rates of enlargement of the different parts of the seed are not equal, for the endosperm may fill the sac tightly at one time and loosely at other times (Plate viii, figs. 13, 16, 17). Similarly the embryo may sometimes, after resorption of the endosperm, be loose in the cavity of the sac.

The mature seed is about 6 × 3·5 × 2 mm. There is considerable shrinkage on drying (Plate viii, fig. 20). The epidermis of the testa is composed of radially elongated, indurated cells which are continued across the insertion of the aril (Text-figs. 50, 51; Plate viii, figs. 16, 17, 19). The testa, though composed of outer integument and funiculus, has no differentiation on its surface.

The columnar cells of the epidermis of the seed are called Malpighian cells (after Malpighi, who first observed them) by Pammel (1899, p. 94), who says they are nearly universal in the Leguminosae. They have been described more recently by Elford (1930, p. 94) in *Albizzia lophantha*, and by Martin (1914, p. 156) in *Trifolium pratense*, while Cook (1924, pp. 443–4) describes the double row of them across the funicular swelling (a cleavage plane) in *Crotalaria sagittalis*. Pammel (loc. cit.) rightly objects to the term "palisade cells", as being likely to cause confusion with palisade mesophyll.

CHANGES WITHIN THE EMBRYO SAC.

General.

Though the primary zygote nucleus is formed before the primary endosperm nucleus, the latter divides first (Text-fig. 58). The sac soon begins to elongate rapidly, separating the zygote from the earlier endosperm nuclei, which appear to be formed in the lower part of the sac (Text-figs. 58, 59; Plate viii, fig. 9). The antipodals quickly degenerate after formation of the primary endosperm nucleus. The growth of the embryo and endosperm does not keep pace with the resorption of the enlarging tissues round them, so that each usually appears within a clear zone of resorption (Plate viii, figs. 13, 16–18). By the time the seed is full size, the embryo has resorbed all the endosperm (Plate viii, fig. 18). The starch disappears from the sac about the 8-nucleate stage of the endosperm.

There is a slight possibility that in the Papilionaceae the division of the zygote and primary endosperm nucleus may be tending to less separation in time, than is the case in the Mimoseae. Weinstein (1926, pp. 257–8) describes the division of the zygote as indefinite in time about the 4- or 8-nucleate endosperm stage in *Phaseolus vulgaris*. Martin (1914) says that in *Trifolium pratense* (p. 158) the endosperm usually precedes the zygote, and in *Vicia americana* (p. 162) the zygote and endosperm nucleus divide about the same time. In *Acacia Baileyana* the zygote does not divide before the 8-nucleate endosperm stage, and Maheshwari (1931, p. 250) records the same for *Albizzia lebbek*.

Pammel (1899, pp. 117–8) quotes many authors who say that there is no endosperm in the mature seed of the Leguminosae. He says that is anatomically not true, for there are usually one or a few layers left, and that *Acacia* seeds, having a much reduced endosperm, are anatomically closely related to those of *Desmanthus* and *Cassia* (p. 216). Weinstein (1926, p. 257) says that the embryo digests almost all the endosperm in the Papilionaceous *Phaseolus vulgaris*. Pammel (1899, p. 216) describes a single persistent layer of endosperm cells (aleurone layer) in *Acacia filicina*. Guignard (1881, p. 45) says that the persistence of the endosperm is variable in the Mimoseae, but it is usually all resorbed in *Acacia*. No trace of endosperm was observed here in the mature seed of *Acacia Baileyana*.

The Endosperm.

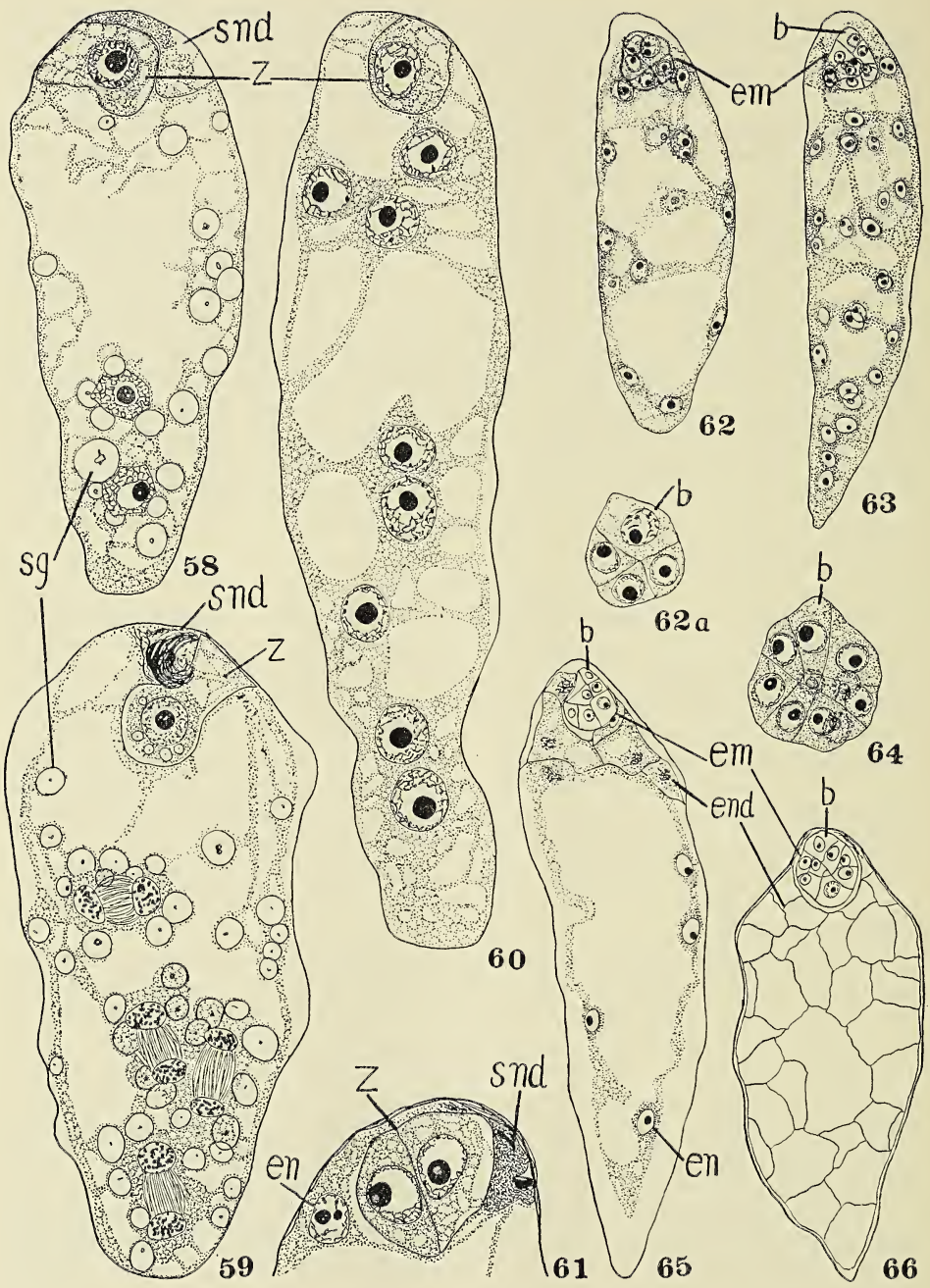
The spindles of the first two divisions of the endosperm appear to have been vertical, judging from Text-figures 58 and 59. The four resulting nuclei take a peripheral position (Plate viii, fig. 9). After the simultaneous division of these nuclei (Text-fig. 59), some of the eight formed collect round the zygote (Text-fig. 60) which is often made difficult of observation in the later stages by the collection of nuclei round it (Text-figs. 62, 63). The divisions of the endosperm nuclei after about the eight-nucleate stage are not always simultaneous. The free endosperm nuclei lie in a peripheral network of cytoplasm that is more closely meshed at the ends of the sac (Text-figs. 62, 63; Plate viii, fig. 12).

Cell formation in the endosperm begins at about the 64-nucleate stage around the embryo of about 12 cells (Text-fig. 65). Tissue formation is basipetal (Plate viii, fig. 10).

In adult or nearly adult endosperms it sometimes appears as if there is the differentiation of a slightly developed "basal apparatus" (cf. Schürhoff, 1926, p. 352 et seq.). Any differentiation that does occur is only incidental and not due to any initial organization of the endosperm nuclei. It is due to the course of cell formation which, as it reaches the base of the sac, sets up temporarily one or more "cells" that are multi-nucleate (Plate viii, fig. 11). Though wall formation finally occurs within these "cells", the common wall remains heavier than the other walls of the endosperm (Plate viii, fig. 13). There is considerable variation in the size of the cells of any one endosperm (Plate viii, figs. 16, 17).

The case shown in Text-figure 66 is unusual, for the endosperm seems to have had centripetal cell formation, and that at a very early stage in the development of the embryo (cf. Plate viii, fig. 10). The ovule itself was small for the stages of development of both the embryo and endosperm.

Schürhoff (1926, pp. 574–9) reviews researches on many species of Leguminosae, and records only "nuclear" endosperm, the type possessed by *Acacia Baileyana*. Guignard (1881, pp. 150–1) says that among the Mimoseae,



Text-figures 58-60.—L.S. embryo sac showing the nucleus of the uni-cellular zygote passing from the resting condition towards prophase, and the formation of eight endosperm nuclei. × 733.

Text-figure 61.—Two-celled zygote originally laterally attached. × 733.

Caesalpineae and the Papilionaceae without or with only a rudimentary suspensor, cell formation in the endosperm begins around the embryo of about twelve cells—as in *Acacia Baileyana*; while among the Papilionaceae with a suspensor, cell formation occurs later and later, till in the Vicieae there is no cell formation in the scanty endosperm and the suspensor serves the nutrient function. Recent researches have extended the absence of cell formation to the Genisteae (*Crotalaria sagittalis*, Cook, 1924, p. 443). In *Colutea arborescens*, Němec (1910) found that the chalazal nuclei of the peripheral layer increased greatly in size and became lenticular; cell wall formation began in the micropylar region and spread to hardly half the cavity of the sac, walls not being generally formed between the large chalazal nuclei. The temporarily multinucleate "cells" at the base of the endosperm of *Acacia Baileyana* suggest the beginning of a trend to the above condition, and through it to the condition in some of the Vicieae.

The Embryo.

The zygote, after its partial approach to division, regains the resting condition which it maintains for a long time. Its nucleus has a large nucleolus lying in a central vacuole. The threads of the peripheral chromatin reticulum are coarse and granular at first (Text-figs. 58, 59), but enter into a spireme soon after the eight-nucleate stage of the endosperm (Text-fig. 60; Plate viii, fig. 12).

The first division of the zygote is by a transverse wall, though if the egg is attached laterally, this wall may appear to be nearly vertical (Text-fig. 61 and Plate viii, fig. 14; cf. Text-figs. 58, 60 and Plate viii, figs. 9, 12). Whatever its original position, the young embryo soon appears at the apex of the sac. The second division is by a wall that is nearly vertical (Text-fig. 62*a*), its slight inclination causing the formation of a cell that has something of the appearance of a basal cell, an appearance that is retained by it or its derivative(s) till just before the formation of the primordia of the cotyledons (Text-figs. 62*a*, 64, and Plate viii, fig. 15).

The young embryo develops by irregular divisions from the quadrant stage to various forms of pear shape. It has no suspensor and is of the massive type (Text-figs. 63–6; Plate viii, figs. 15, 16). Plate viii, figure 17, shows the young cotyledons and between them the region whence the plumule will arise, the place and time of origin of the primordial meristems being indefinite.

In the mature seed the embryo occupies all the cavity within the testa except for a small residue of the chalaza and perhaps some liquid of resorption (Plate viii, fig. 18). The plane of the cotyledons is parallel with the broad and long axes of the seed, and is perpendicular to that shown for the cotyledon primordia. It is not known whether this difference of orientation is a frequent feature or not; the similar orientation of young cotyledons to that shown for the old has been seen. The cotyledons are spurred (Text-figs. 52, 54; Plate viii, fig. 18) and have the vascular bundles on the inner (adaxial) face (Text-figs. 52, 56, 57).

Text-figures 62, 63.—Sacs with 16 and 32 peripheral nuclei and 4- and 14-celled embryos (only 6 cells shown in embryo of 63). × 236.

Text-figures 62*a* and 64.—4-celled embryo from 62 and an embryo (not all cells shown) at the stage of about 60 endosperm nuclei. × 453.

Text-figure 65.—L.S. Sac showing the beginning of cell-wall formation around the embryo. × 236.

Text-figure 66.—L.S. Sac showing result of apparently centripetal wall formation in the endosperm of a diminutive seed. × 236.

The radicle is very broad and blunt, with convex sides and well differentiated meristems, but no root cap. The plumule is well advanced in organization, having the pinnae formed on the first leaf (Plate viii, fig. 19) and the primordia of three or four other leaves present (not shown in this figure).

The vasculation of one embryo was examined in detail. In the radicle the stele was tetrarch and approximately square in cross-section, with a side of the square parallel with the plane of the cotyledons (Text-figs. 53–5). In the region of the hypocotyl the bundles in each corner appeared paired. The bundles for the cotyledon arose from the adjacent members of the pairs at the corners on that side (Text-fig. 56). Soon after leaving the stele, each member of the pair of bundles for the cotyledon bifurcated. The adjacent arms of the bifurcations joined to form the midrib of the cotyledon (Text-figs. 52, 56, 57). The distant arms of the bifurcation each give rise to the three lateral and the one spur primary bundles on their respective sides of the cotyledon (Text-figs. 52, 56, 57). Secondary bundles anastomosed between the seven primary ones. The orientation of the stele in the plumule was such that a diagonal of its square outline is parallel with the plane of the cotyledons.

The transverse division of the zygote is universal in the Leguminosae (Guignard, 1881, p. 142), the second division being vertical in the Mimoseae, but, as exampled by *Acacia retinoides*, the walls in the upper and lower halves are at right angles (p. 29). The second division is in the one plane in *A. Baileyana*. The young embryo described by Guignard is very similar to that described above. In common with the other Mimoseae, *A. Baileyana* has not even a rudimentary suspensor. This is in contrast with the Rosaceae (Péchoutre, 1902, p. 155) and the greater number of the Papilionaceae (Guignard, 1881, pp. 142 et seq.).

That the radicle is without a root cap in the mature seed is in line with the observations of Kater (1927, p. 632) on *Phaseolus vulgaris*, in which plant the root cap comes with the first onset of cell division in germination. Guignard (1881, p. 33) comments on the two forms of embryo axis (radicle) in the Acacias. That of *Acacia farnesiana* is of uniform thickness, and that of *A. decurrens*, *retinoides*, *melanoxylon*, *brachybotrya*, and *exudans* bulges in the middle; also these latter species have auriculate cotyledons. *Acacia Baileyana* is of the second type. It is to be noted that among these species mentioned, *A. farnesiana* is the only one that is not endemic to Australia.

The quadrangular stele of the one embryo axis figured is in constrast with the 2/5 phyllotaxy of the leaves, the 5-rayed pedicel of the flowerhead and the 2/5 phyllotaxy of the flowers described in Part 1 (Newman, 1933*b*, pp. 148–150). Compton (1912*b*, p. 100) says the tetrarch hypocotyl is characteristic of the Leguminosae in general and the Caesalpineae and Mimoseae in particular. He gives figures of *Acacia doratoxylon* seedling, but does not show the twist in the stele found in this one of *A. Baileyana*.

Seed Germination.

Mature Seeds.

The cells of the cotyledons of the green seeds are large and contain a little starch in small grains. The cells of the dry seeds contain a large quantity of small starch grains. The difficulty of staining with iodine indicates that the starch in both conditions may not be quite normal. The drying of the seed produces a considerable contraction (Plate viii, fig. 20). The outer layer of the testa of the green seed is indurated; on drying, it becomes black. The swelling on

the funiculus (aril) does not shrivel away in the dry seed, and maintains some of its green colour for some time (the dark area on it in the dry seeds of Plate viii, fig. 20).

Acacia Baileyana is like other Acacias in the hardness and longevity of its seeds. Very drastic means are sometimes taken to produce rapid germination. Myers and Liels (1932, p. 947) made germination tests with seeds of *Acacia Baileyana*, and, by the use of boiling water before sowing, obtained 92% germination in 20 days, as against 4% germination of untreated seeds.

Dormancy and Variability.

It is well known that *Acacia Baileyana*, in common with many other species of *Acacia*, shows a high degree of variability in cultivation and in nature. This variation is usually attributed to hybridization, but in view of the following considerations some of it may arise as mutation during dormancy.

Nawaschin (1933) found that prolonged dormancy of the seeds was responsible for a tremendous increase in the rate of chromosomal mutations in *Crepis tectorum*. After rejecting selective mortality as the cause of increased rate of mutation, he say: "Secondly, the observed increase in the mutation rate cannot be attributed to accumulation of the direct effect of some external agency like radiation and the like; for the rate of mutants was shown not to be proportional to the length of the period of 'rest', but, on the contrary, must have grown with an enormous and progressive velocity until after five years it was a thousand times as great as after one year. One is forced thereby to the conclusion that the main agency that caused spontaneous chromosomal mutations should be sought not outside, but rather inside the cell. *The same would probably also hold true for factor mutations*" (italics mine). Now Kater (1927, p. 634) describes the nuclear changes in the attainment of the dormant condition in *Phaseolus vulgaris*. The stainable chromatin appears to collect in the much enlarged, central nucleolus, with very faint threads radiating from it. Earl (1927, p. 69), in a microchemical study of the nucleus in *Vicia faba*, supports the idea of the chromosome as essentially an ultramicroscopic thread of "genes" surrounded by a chromatic matrix, "the visible chromosome" that waxes and wanes from time to time and is the immediate environment of the genes (p. 71). If the observations of Kater and Earl are valid, then in the cells of dry seeds (dormant) the "gene" threads are more exposed to the influence of the intra-cellular environment than in non-dormant cells. This suggests an explanation of both the great increase in mutation rate in seedlings from long dormant seeds, and the rapidity with which that rate increases on account of the delicacy and unprotected state of the "gene" threads.

The Form of the Seedling.

The cotyledons are raised above the ground by the hypocotyl, which is from two to five centimetres long (Cambage, 1917, p. 391, records one 10·5 cm.). The first leaf is simply pinnate, all of 75 seedlings raised showing this feature. All of 49 seedlings that survived to the second leaf had this leaf bipinnate with one pair of pinnae (cf. Cambage, 1915, pp. 82–83). After the second leaf, the time and continuity of the increase in the number of pairs of pinnae vary considerably.

The first leaf was observed to be nyctitropic by closing together of the pairs of pinnae. Cambage has described such behaviour among the species of *Acacia* (1925, p. 230; 1926, p. 85).

The sequence of the seedling leaves of Acacias has been taken as indicating the origin of the bipinnate forms from pinnate forms (Cambage, 1915, p. 85). Kelly (1912, p. 117) propounds a contrary theory that the bipinnate form "is highly specialized, and is evolved from the entire form by gradation through the serrate, crenulate, and lobed forms, until the great surface aggregate of doubly-feathered leaves is attained". Zimmerman (1930) derives the bipinnate form through the pinnate form by a process of condensation from a system of alternate simple leaves (cf. Newman, 1933*a*, p. 140). Kelly (p. 122) does, however, suggest a process of condensation in the formation of the whorls of the leaves of *Acacia verticillata*. His theory of the bipinnate leaf does not account for the presence of radial steles in the rachis of the feathery leaves of many Acacias (Peters, 1927).

The condensation theory does account for both the radial steles of the adult rachis and the sequence of the seedling leaves.

Chronology and Synchronology.

This section covers the whole of the life history of *Acacia Baileyana*.

chronology.

The times stated here are for the trees at Hill Top. The young racemes appear in December, in the middle of summer. The flowers then initiated come to perfection in June and July, the depth of winter. By the end of March the young racemes are about five centimetres long, and some of the flowerheads would be nearly half size. Heads of half size have microspore germination completed and the germination of the megaspore in progress. By the end of May it is difficult to collect good stages of sporogenesis. In May there are many heads full size. Anthesis begins early in June, and continues through July, occurring during the coldest period of the year. Material collected between 1st July and 25th August showed stages from sperms present to completion of triple fusion, carpels being pink and up to 3 mm. long by the end of the process. The time taken seems long and variable. Four-nucleate endosperm was found in carpels between 6 and 12 mm. long, eight-nucleate endosperm in carpels between 3 and 6 mm. long, and sixteen-nucleate endosperm in carpels more than 25 mm. long, all collected on 22nd September. In harmony with the above, the time taken for cell formation in the endosperm seems variable. The seeds are full size early in December, but the pods have attained full size early in November. Dehiscence of the pods is in the latter half of December, one year after the initiation for flower primordia. For the Sydney district the times would be up to one month earlier.

synchronology.

The general impression gained from the course of the investigation is that both absolute and relative times are subject to variation. The following columns give the approximate synchrony of the phases of development.

Microsporogenesis and male gametogenesis.	Megasporogenesis and female gametogenesis.
Filament and anther and archesporium.	Ovule primordium.
Four sporogenous cells.	Archesporium.
Meiosis.	Primary sporogenous cell.
Germination of the spore.	Meiosis.
	Germination of the spore.

Fertilization.

Embryo.	Endosperm.	Ovule.
Fusion of nucleoli and regained resting condition.	Triple fusion.	Outer integument near top of embryo sac.
	Division of primary nucleus.	Micropyle completed.
Division of zygote.	8-nucleate.	

From here on the variation is so great that it is impossible to correlate the times of the various stages.

Conclusion.

The detailed investigation just concluded of the life history of *Acacia Baileyana*, has been carried out to provide a basis of comparison for the wider study of the genus *Acacia*. Apart from its bearing on the comparative study, this inquiry has touched on several questions of independent interest, which will receive further attention in the general course of the Studies in the Australian Acacias. These questions are:

1. The relation of the last pre-meiotic division to chromosome pairing; for, in the Acacias, it is possible to determine precisely which is the last pre-meiotic mitosis in microsporogenesis and megasporogenesis.
2. The chromatin changes during fertilization.
3. The possibility of the occurrence of polyspermy in connection with endosperm formation.
4. The effect of prolonged dormancy on mutation rate.

In the comparative aspect of the inquiry, it is not possible to draw any definite conclusions, for the evidence seems to be rather confused. A great amount of further research and of study of literature will be necessary for that purpose. It is, however, desirable to state where the confusion exists, that the way may be prepared for its resolution.

It is generally assumed that in the Rosales the Mimosa-tribe and the Rosa-tribe are closely related and primitive, and that from the Mimoseae the line of evolution in the Leguminosae was through the Caesalpineae to the Papilionaceae. We thus expect that if any characters are shared, it will be more primitive ones between the Mimoseae and Caesalpineae, and more advanced ones between the Caesalpineae and Papilionaceae. While we would not expect any sharing of characters between the Mimoseae and Papilionaceae that were not also possessed by the Caesalpineae. But it does not appear so simply as this in fact.

The Mimoseae and Papilionaceae have the following characters in common: 1. Uni-nucleate tapetal cells in the anther (primitive); 2. Delayed polar fusion (advanced); 3. Non-persistence of the inner integument in the seed (advanced); 4. The more anatropous ovule (advanced). Of these characters *Acacia Baileyana* shows the first three and is indefinite with regard to the fourth.

The Mimoseae and Caesalpineae have the following characters in common: 1. Persistent antipodals (primitive); 2. Simple vasculation of the seed; 3. Tetrarch

hypocotyl; 4. Only three and four equal megaspores (primitive); 5. Mode of cell formation in the endosperm (less advanced), shared with some Papilionaceae; 6. No suspensor, shared with some Papilionaceae; 7. Uni-cellular megarchesporium (advanced). *Acacia Baileyana* shows all these characters, but is slightly modified in 4 and 5.

Other independent features manifested by *Acacia Baileyana* are: 1. More than one pollen tube to one embryo sac (possibly primitive); 2. Delay in the growth of the integuments (advanced); 3. Secondary polyploidy (for the Acacias in general, an advanced condition over the genera having the simple basic numbers).

These considerations show that it is impossible as yet to make any pronouncement on the systematic position of *Acacia Baileyana* in the Leguminosae. They emphasize the need for more work of this kind for the elucidation of that position and for giving a fuller understanding of the systematic arrangement of the Natural Order itself.

Summary.

This paper concludes the study of the life history of *Acacia Baileyana*.

The microspores are thickened only on the face toward the outside of the pollinium. Their germination in the anther begins with vacuolation of the cytoplasm, and produces the generative cell and tube nucleus. The generative cell with chromosomes organized is spindle-shaped. The cytoplasm of the generative cell is unusual in that it is less dense than the surrounding cytoplasm.

Anthesis is proterogynous, but there is no precise method for pollination, which occurs in the depth of winter.

Germination of the pollen, whether occurring naturally or in culture, is towards the inside of the pollinium which is thereby split; and through the slits the tubes emerge, with the generative cell usually preceding the disorganized tube nucleus. Two slightly elliptical male nuclei (showing chromosomes) are discharged into the sac. Methods of pollen culture are discussed and it was found that an agar medium of 20% cane sugar and 1% agar gave best results. The pollen seems at its best about three or four days after anthesis. The path of the pollen tubes is described and it is recorded that more than one can attack one embryo sac. The empty pollen tube appears to be hardened in the ovary, and persists for a long time projecting from the tip of the ovule which is naked at fertilization. The disturbance of the contents at the top of the embryo sac is variable. There is a discussion of the entry of more than one pollen tube into one embryo sac.

Germination of the megaspore begins with vacuolation, and the usual three nuclear divisions result in the eight-nucleate sac, in which are formed later the cells of the egg apparatus and antipodal group; the egg and upper polar are regarded as probably sisters. Starch appears first in the megaspores and is present during the formation of the adult embryo sac, soon after the completion of which it fills the whole cavity of the sac, obliterating the central vacuole and obscuring the structures in the sac by its abundance. It is within the cells of the egg apparatus and antipodal group. It does not react normally with the usual reagents. The egg may be beside or below the hooked synergids, otherwise the egg apparatus is normal. The polars are unfused at fertilization. The antipodals are cells and disintegrate soon after triple fusion. The enlargement of the developing sac obliterates almost all of the parietal tissue. There is discussion of starch in the embryo sac, hooked synergids, and the time of polar fusion.

The male gametes that begin fertilization are naked nuclei containing telophase chromosomes. As fusion approaches they attain the resting condition. The egg and polar nuclei are also resting at the time of fusion, which occurs first with the egg. In the formation of the primary endosperm nucleus, fusion is first between the male and one polar and then the other polar nucleus. After fusion of the nuclei, a small extra nucleolus appears in the "egg" and "polar" nuclei, and is attributed to the male nucleus. In the zygote the nucleoli fuse, and after a partial approach to prophase the nucleus enters the resting condition. There is probably no fusion of nucleoli in the primary endosperm nucleus which divides much earlier than the zygote. There is discussion of the literature relevant to these phenomena of fertilization.

From the number of pollen tubes in excess of the ovules that enter the ovary, the fact that two tubes have been seen in several instances to attack one embryo sac, the presence in some cases of one or more small, extra nucleoli in association with the polar nuclei, and certain abnormal chromosome numbers in some endosperms, it is deduced that occasional polyspermy in connection with endosperm formation is probable. The relevant literature is discussed at length.

Post-fertilization changes in the carpel and ovule tissues are described. It is only after fertilization that the integuments develop beyond primordia, the micropyle being formed round the projecting remains of the pollen tube. There is a single vascular bundle in the outer integument. A cushion-like aril is developed on the funiculus. The epidermal layer of the testa is composed of malpighian cells.

The primary endosperm nucleus divides before the zygote. The endosperm resorbs the parietal tissue and much of the chalazal tissue and almost all of the lateral tissue of the nucellus. The embryo resorbs all the endosperm. There is free nuclear division in the endosperm till there are about 64 nuclei in the peripheral cytoplasm of the sac. At about that stage, cell formation begins round the 12-celled embryo and proceeds basipetally, forming at the base temporarily multinucleate cells, which simulate a slight "basal apparatus".

The first division of the zygote is horizontal, the second is almost vertical. There is no basal cell or suspensor. The primordial meristems are indefinite in origin. The development and vasculation of the cotyledons is described. The first leaf has the pinnae clearly shown in the adult green seed.

The mature seed, its germination and the form of the seedling are described, with discussion of recapitulation in the seedling leaves and of dormancy and variability.

The seasonal stages of the development are recorded and the relative times of the lines of development in the flower are set out in a table.

In conclusion, it is pointed out that several questions of general cytological interest arise for further investigation; and that the inquiry does not allow of any definite statement on the systematic position, but points to the need for further work of this kind for its elucidation.

I would express my thanks to Professor T. G. B. Osborn for the facilities provided in this department and for his interest in the work.

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NOTES ON THE ILLUSTRATIONS.

All the Text-figures have been prepared from camera-lucida drawings. All the figures on Plate viii are from photographs. The illustrations are the work of the writer. All magnifications have been obtained by measurement.

Annotations have been standardized throughout the Text-figures and Plate-figures. A few special letterings are given in the legends and explanations. The following are the standard letterings:

at. Antipodals; *ap.* Antipodal pocket; *b.* Cell simulating basal cell; *c.* Region of crushing or resorption; *cot.* Cotyledon; *cs.* Cotyledon spur; *e.* Egg; *ea.* Egg Apparatus; *em.* Embryo; *EN.* Primary endosperm nucleus; *en.* Endosperm nucleus; *end.* Cellular endosperm; *es.* Embryo sac; *ex.* Extine; *f.* Funiculus; *fs.* Funicular swelling (Aril); *g.* Generative cell; *gr.* Groove in extine; *in.* Intine; *ii.* Inner integument; *m.* Malpighian cells; *mi.* Micropyle; *mn.* Male nucleus; *mnl.* Male nucleolus; *o.* Outgrowth of the funiculus to complete the micropyle; *oi.* Outer integument; *pn.* Polar nucleus; *pnl.* Polar nucleolus; *pt.* Pollen tube; *sg.* Starch grain(s); *sn.* Synergid(s); *snd.* Synergid disintegrating; *SP.* Functional megaspore; *sp.* Non-functional megaspore(s); *tn.* Pollen tube nucleus; *vb.* Vascular bundle; *z.* Zygote.

EXPLANATION OF PLATE VIII.

Figures 1-5.—Germination of pollinia, showing (except 3) germination of the grains towards the inside of the pollinium. 1, 2 and 4 are from sections. 1, On a stigma, × 566; 2, In culture, showing the exit of the generative cell, and the wall of the tube continuous with the intine, × 530; 3, Two pollinia on one stigma, × 500 (approx.); 4, On a stigma, showing the wall of the tube continuous with the intine, × 530; 5, In culture showing the bursting of the pollinia and exit of the tubes, × 175 (approx.).

Figure 6.—Oblique L.S. nucellus with germinating distal megaspore, having the central vacuole plasmolysed, × 434.

Figure 7.—Side view of a three-armed metaphase plate in an abnormal endosperm, × 800.

Figure 8.—Slightly oblique section of a definitive embryo sac, showing the striated apical cytoplasm of the synergids, the nucleoli of the polar nuclei, and the abundance of starch obscuring the limits of the structures in the sac, × 530.

Figures 9-14.—Division of the zygote and development of the endosperm, from longitudinal sections of ovules. 9, Nucellus (showing multiple epidermis) and inner integument at the stage of the 4-nucleate endosperm; zygote undivided, × 240; 10, Embryo sac with bounding layer of nucellus. The upper part of the endosperm is cellular, the lower still has only peripheral nuclei, the embryo is about to form cotyledon primordia, × 250; 11, Lower part of an endosperm showing a multinucleate cell (*x*) at the base, × 132; 12, Whole ovule showing three of eight endosperm nuclei, the undivided zygote, and the micropyle in the inner integument (just missed by the section in the outer integument), × 110; 13, Part of the chalaza and lower part of the endosperm after cell formation in the temporarily multinucleate cells. Note elongated cells of the chalaza leading up to the antipodal pocket, × 120; 14, Nucellus and inner integument, showing three of sixteen endosperm nuclei, and the two-celled zygote, × 240.

Figure 15.—L.S. young embryo (from Fig. 16) showing cells simulating basal cells. Before origin of cotyledons, × 110.

Figures 16-17.—L.S. in the broad plane of young seeds before and after the origin of the cotyledons, × 27·5.

Figure 18.—L.S. in the narrow plane of a full-grown seed, showing the plumule, radicle, and cotyledons (spurred). The micropylar region and the aril were broken off, × 8.

Figure 19.—L.S. upper part of a full-grown seed, parallel with the cotyledons, showing the radicle, plumule with pinnae (*pi*) on the first leaf, and the aril, × 12.

Figure 20.—Showing the contraction of the seeds on drying. Four sizes from the largest to the smallest of both green and dry seeds were selected and arranged in the broad and narrow aspects. The two left-hand rows are the green seeds, the two right-hand rows are the dry seeds. About natural size.